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INFECTIONS IN LUNG AND HEART TRANSPLANT RECIPIENTS

- Studies on the impact of bronchoscopy in the diagnosis of respiratory infections and detection of viral infections in blood and bronchoalveolar lavage fluid

Juho Lehto

Academic Dissertation

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Wisdom is the principal thing, therefore get wisdom; and with all thy getting, get understanding.

Proverbs 4:7

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LIST OF ORGINAL PUBLICATIONS

This thesis is based on the following original publications which are referred to in the text by their Roman numerals.

I Lehto JT, Anttila V-J, Lommi J, Nieminen MS, Harjula A, Taskinen E, Tukiainen P, Halme M. Clinical usefulness of bronchoalveolar lavage in heart transplant recipients with suspected lower respiratory tract infection. The Journal of Heart and Lung Transplantation 2004;23:570-576.

II Lehto JT, Koskinen PK, Anttila V-J, Lautenschlager I, Lemström K, Sipponen J, Tukiainen P, Halme M. Bronchoscopy in the diagnosis and surveillance of respiratory infections in lung and heart-lung transplant recipients. Transplant International 2005;18:562-571.

III Lehto JT, Lemström K, Halme M, Lappalainen M, Lommi J, Sipponen J, Harjula A, Tukiainen P, Koskinen PK. A prospective study comparing cytomegalovirus antigenemia, DNAemia and RNAemia tests in guiding pre-emptive therapy in thoracic organ transplant recipients. Transplant International 2005;18:1318-1327.

IV Lehto JT, Halme M, Tukiainen P, Harjula A, Sipponen J, Lautenschlager I. Human herpesvirus-6 and -7 after lung and heart-lung transplantation. The Journal of Heart and Lung Transplantation. In press.

ABBREVIATIONS

ATG	Antithymocyte globulin
AUC	Area under the curve
AZA	Azathioprine
BAL	Bronchoalveolar lavage
BALF	Bronchoalveolar lavage fluid
BOS	Bronchiolitis obliterans syndrome
CAV	Cardiac allograft vasculopathy
CD	Cluster of differentiation
cFB	Clinically indicated flexible bronchoscopy
CMV	Cytomegalovirus
COPD	Chronic obstructive pulmonary disease
Cs	Corticosteroids
CT	Computed tomography
CyA	Cyclosporine A
D	Donor
DNA	Deoxyribonucleic acid
EBV	Ebstain-Barr virus
EDTA	Ethylenediaminotetra-acetic acid
FB	Flexible broncoscopy
GAN	Ganciclovir
HHV-6	Human herpesvirus-6
HHV-7	Human herpesvirus-7
HHV-8	Human herpesvirus-8
HLTR	Heart-lung transplant recipient
HLTx	Heart-lung transplantation
HRCT	High-resolution computed tomography
HSV	Herpes simplex virus
HTR	Heart transplant recipient
HTx	Heart transplantation
IFN-γ	Interferon-γ
IL-2	Interleukin-2
IP	Inhaled pentamidine
ISHLT	International Society for Heart and Lung Transplantation
LTR	Lung transplant recipient
LTx	Lung transplantation
MMF	Mycophenolate mofetil
mRNA	Messenger ribonucleic acid
NASBA	Nucleic acid sequence-based amplification
NF-κB	Nuclear factor-κB
OB	Obliterative bronchiolitis
OKT-3	Muromonab-CD3 (monoclonal antibody to CD3)
PBMC	Peripheral blood mononuclear cell
PCP	*Pneumocystis carinii* pneumonia
PCR	Polymerase chain reaction
PMNL	Polymorphonuclear leukocyte
POD	Postoperative day
PPV	Positive predictive value

PSB	Protected specimen brush
PTLD	Post-transplant lymphoproliferative disorder
R	Recipient
RNA	Ribonucleic acid
ROC	Receiver-operating characteristic
RSV	Respiratory syncytial virus
sFB	Surveillance flexible bronchoscopy
SIR	Sirolimus
SMC	Smooth muscle cell
SOT	Solid organ transplantation
Tac	Tacrolimus
TBB	Transbronchial lung biopsy
TMP-SMZ	Co-trimoxazole (Trimethoprim-Sulfamethoxazole)
TNF-α	Tumour necrosis factor α
Tx	Transplantation
valGAN	Valganciclovir
VZV	Varicella zoster virus

ABSTRACT

Infection is a major cause of mortality and morbidity after thoracic organ transplantation. The microbiological aetiology of the infections is broad necessitating accurate diagnostic evaluation. The aim of the present study was to evaluate the infectious complications after lung and heart transplantation, with a special emphasis on the usefulness of bronchoscopy and the demonstration of cytomegalovirus (CMV), human herpes virus (HHV)-6, and HHV-7.

We reviewed the diagnoses established from the specimens of all consecutive bronchoscopies performed on heart transplant recipients from May 1988 to December 2001 (n = 44) and lung transplant recipients from February 1994 to November 2002 (n = 472). To compare different assays in the detection of CMV and guiding the antiviral therapy, a total of 21 thoracic organ transplant recipients were prospectively monitored by CMV pp65-antigenemia, DNAemia (PCR), and mRNAemia (NASBA) tests. The antigenemia test was the reference assay for therapeutic intervention. In addition to CMV antigenemia, 22 lung transplant recipients were monitored for HHV-6 and HHV-7 antigenemia.

The overall diagnostic yield of the clinically indicated bronchoscopies was 41 % in the heart transplant recipients and 61 % in the lung transplant recipients. The utility of the bronchoscopy was highest from one to six months after transplantation. In contrast, the findings from the surveillance bronchoscopies performed on lung transplant recipients led to a change in the previous treatment in only 6 % of the cases. *Pneumocystis carinii* and CMV were the most commonly detected pathogens in the bronchoscopic specimens. Furthermore, 15 (65 %) of the P. carinii infections in the lung transplant recipients were detected during adequate chemoprophylaxis. Although some complications of the bronchoscopy were detected, none of them were fatal.

Antigenemia, DNAemia, and mRNAemia were present in 98 %, 72 %, and 43 % of the CMV infections detected in the study population, respectively. The optimal DNAemia cut-off levels (sensitivity/specificity) were 400 (75.9/92.7 %), 850 (91.3/91.3 %), and 1250 (100/91.5 %) copies/ml for the antigenemia of 2, 5, and 10 pp65-positive leukocytes/50 000 leukocytes, respectively. The sensitivities of the NASBA were 25.9, 43.5, and 56.3 % in detecting the same cut-off levels of antigenemia. In general, CMV DNAemia was detected in 93 % and mRNAemia in 61 % of the CMV antigenemias requiring antiviral therapy. HHV-6, HHV-7, and CMV antigenemia was detected in 20 (91 %), 11 (50 %), and 12 (55 %) of the 22 recipients (median 16, 31, and 165 days) after lung transplantation, respectively. HHV-6 antigenemia occurred in 15 (79 %) and HHV-7 antigenemia in seven (37 %) of these patients during ganciclovir or valganciclovir prophylaxis, while 11/12 of the CMV antigenemias were delayed beyond the cessation of prophylaxis. One case of pneumonitis and another of encephalitis were associated with HHV-6, but no other clinical manifestations could be linked to HHV-6 or HHV-7.

The results of the present study indicate that bronchoscopy is a safe and useful diagnostic tool in lung and heart transplant recipients with a suspected respiratory infection, but the role of surveillance bronchoscopy in lung transplant recipients remains controversial. The Cobas PCR assay acts comparably with the antigenemia test in guiding the pre-emptive therapy against CMV when threshold levels of over 5 pp65-antigen positive leukocytes are used. In contrast, the low sensitivity of NASBA limits its usefulness in the guidance of the pre-emptive therapy. HHV-6 and HHV-7 activation is common after lung transplantation, but immediate clinical manifestations are infrequently linked to them. Antiviral prophylaxis against CMV is not able to prevent the appearance of HHV-6 and HHV-7 antigenemia. Future studies are needed to evaluate the overall efficacy of the surveillance bronchoscopies and preventive antiviral strategies on CMV, HHV-6, and HHV-7 taking into account both the direct and indirect effects of infections.

INTRODUCTION

The first successful lung transplantation (LTx) was performed by James Hardy in 1963 and the first heart transplantation (HTx) by Christian Barnard in 1967 (Hardy et al. 1963, Barnard et al. 1967). The early results were poor, and it was not until the 1980's by the discovery of cyclosporine (CyA) when lung, heart, and heart-lung transplantation (HLTx) gained a widespread acceptance as a therapeutic option. Today transplantation (Tx) offers markedly improved quality and longer expectancy of life for patients suffering from end-stage heart or lung disease (Arcasoy and Kotloff 1999). Approximately 1800 lung and 3000 heart transplants are reported annually to the registry of the International Society for Heart and Lung Transplantation (ISHLT) (Taylor et al. 2006, Trulock et al. 2006). Despite the improvement on the outcomes, important postoperative complications still lower the five-year survival rate of lung transplant recipients (LTRs) and heart transplant recipients (HTRs), being approximately 49 % and 68 %, respectively (Taylor et al. 2006, Trulock et al. 2006).

Infection is a major complication of LTx and HTx limiting the survival of the recipients during the first postoperative year (Speich and van der Bij 2001, Taylor et al. 2006, Trulock et al. 2006). This is mainly due to the immunosuppressive therapy impairing the host defenses but, to some extent, also to the continuous exposition of the allograft to the external environment in LTRs. The aetiology of the infections in transplant recipients is broad and differs from that of the general population, which makes the empiric therapy without a definite diagnosis difficult and hazardous. Thus, accurate diagnostic evaluation, when an infectious complication is suspected, is of great importance. The most common site of infection in both LTRs and HTRs is the respiratory tract, and diagnostic bronchoscopy is recommended as the initial invasive procedure in order to achieve specimens for microbiological investigations (Speich and van der Bij 2001, Miller et al. 1994, Nusair and Kramer 1999). However, only very few studies on the diagnostic usefulness of

bronchoscopy in HTRs exist, and the role of bronchoscopy in the surveillance of infections and other postoperative complications in asymptomatic LTRs is a matter of debate (Schulman et al. 1988, Valentine et al. 2002).

Cytomegalovirus (CMV) remains a significant cause of morbidity and mortality in thoracic organ transplant recipients despite advances in the development of antiviral agents (Rubin 2000, Zamora 2004a). In addition to the short-term morbidity and mortality of the CMV infection itself, activation of the virus is also associated with chronic allograft injury (Zamora 2004a, Valantine 2004). A convenient, reliable, and rapid test is needed to detect the CMV infection. It would guide the use of antiviral agents and thereby prevent the complications of CMV in transplant recipients. CMV belongs to the beta-herpesvirus family, together with human herpesvirus-6 (HHV-6) first isolated in 1986 and human herpesvirus-7 (HHV-7) found in 1990 (Salahuddin et al. 1986, Frenkel et al. 1990). After primary infection during early childhood, HHV-6 and HHV-7, as other herpesviruses, can establish a latent infection for lifetime and reactivate during immunosuppression. Despite the increasing number of studies detecting HHV-6 and HHV-7 in transplant recipients, the clinical significance of these viruses is poorly understood and LTRs are included only in very few studies (Emery 2001, Jacobs et al. 2003).

The present thesis was designed to study the diagnostics of infectious complications after LTx and HTx with a special emphasis on the usefulness of bronchoscopy and the demonstration of CMV, HHV-6, and HHV-7 infections.

REVIEW OF THE LITERATURE

1. Lung and heart transplantation

1.1. Indications and outcome

Transplantation for the end-stage lung or heart disease is generally considered when a patient suffers from severe symptoms limiting daily living activities (New York Heart Association class III or IV) despite all optimal medical therapy available, and the survival is expected to be less than 2-3 years. The severity of the heart or lung disease has to meet the established criteria for Tx, but the functional status of the patient has to be good enough to allow successful LTx or HTx (Costanzo et al. 1995, Massad 2004, Orens et al. 2006). In addition, contraindications for thoracic organ Tx, such as active malignancies, serious dysfunction of other organs (e.g. kidney, liver, heart, and lung), active and uncontrolled infection, progressive neuromuscular disease, unresolved psychosocial problems, smoking (LTx) or other substance abuse should not be present (Massad 2004, Orens et al. 2006). Heart-lung transplantation may be offered to patients with a congenital heart disease and end-stage lung disease which cannot be otherwise cured (Trulock 1997). The indications for LTx, HTx, and HLTx are presented in Table 1.

Table 1. Indications for lung, heart, and heart-lung transplantation.

	Lung (%)		Heart (%)		Heart-lung (%)	
Diagnosis	ISHLT	Finland	ISHLT	Finland	ISHLT	Finland
COPD / Emphysema	38	14			4	7
Idiopathic pulmonary fibrosis	19	25			3	3
Cystic fibrosis	16	4			16	-
α1-antitrypsin deficiency	8	30			2	-
Primary pulmonary hypertension	4	10			24	40
Bronchiectasis	3	3			1	
Sarcoidosis	3	-			1	-
Re-transplantation	2	3	2	0.3	2	-
Lymphangioleiomyomatosis	1	5				
Congenital heart disease	1	3	2	2	32	40
Cardiomyopathy			46	56		
Coronary artery disease			42	33		
Valvular heart disease			3	5		
Acquired heart disease					4	10
Other	5	4	5	4	10	-

Data from the registry of the ISHLT (www.ishlt.org/registries) and from Helsinki University Central Hospital;
COPD, chronic obstructive pulmonary disease; ISHLT, International Society for Heart and Lung Transplantation.

Actuarial survivals after LTx and HTx are shown in Figure 1. The long-term survival after LTx is shorter compared to the results of HTx and other solid organ transplantation (SOT). The high occurrence of rejection and infection in LTRs is probably the main factor responsible for the earlier allograft injury after LTx (Studer et al. 2003). However, the survival rate of LTRs operated after the mid-1990's has improved compared to that of the earlier years of LTx (Trulock et al. 2006). Approximately 85 to 90 % of the survivors have no activity limitations five years after LTx and HTx, and the recipients report improved and acceptable quality-of-life after Tx (Vermeulen et al. 2003, Karam et al. 2003, www.ishlt.org/registries).

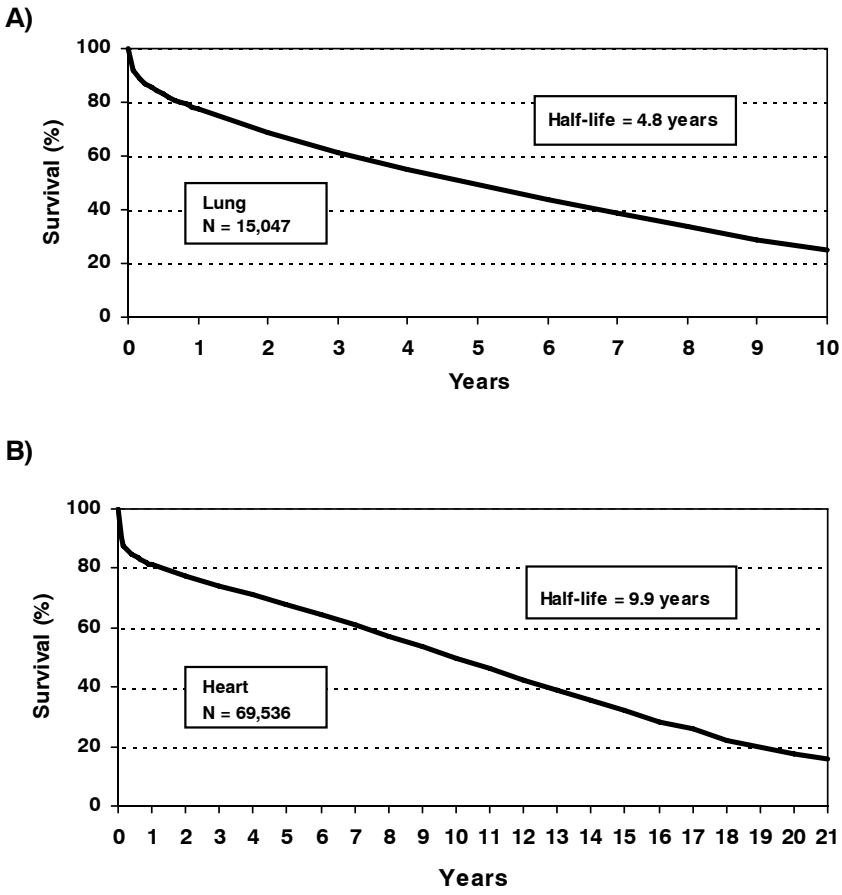

Figure 1. Acturial survival after lung (A) and heart (B) transplantation (www.ishlt.org/registries).

1.2. Immunosuppressive therapy

Life-long medication is mandatory for transplant recipients in order to suppress the host alloimmune responses against the donor organ and thereby to prevent rejection of the allograft. In the vast majority of LTRs and HTRs this immunosuppressive therapy consists of calcineurin inhibitor (cyclosporine A (CyA) or tacrolimus (Tac)), purine synthesis inhibitor (azathioprine (AZA) or mycophenolate mofetil (MMF)), and corticosteroids (Cs) (Taylor et al. 2006, Trulock et

al. 2006). Calcineurin inhibitors suppress T-lymphocyte activation and proliferation by blocking the interleukin(IL)-2 gene transcription, AZA and MMF inhibit T and B lymphocyte proliferation by interfering the purine synthesis, and Cs have multiple anti-inflammatory effects mediated through the inhibition of RNA, DNA, and protein synthesis (Knoop et al. 2003, Lindenfeld et al. 2004). Sirolimus and its derivative everolimus are new immunosuppressive drugs, which may offer some advances to HTRs and LTRs (e.g. antiproliferative effects on chronic allograft injury), but their overall usefulness is not yet clear (Mancini et al. 2003, Knoop et al. 2003). In addition to the maintenance immunosuppression, approximately 40-50 % of thoracic organ transplant recipients receive perioperative induction therapy with anti-lymphocyte/thymocyte globulin or IL-2-receptor antibodies (Taylor et al. 2006, Trulock et al. 2006). The immunosuppressive drug-combinations used in HTRs and LTRs are listed in Table 2.

Table 2. Immunosuppressive therapy at one year post-transplant in lung and heart transplant recipients.

Drugs [a]	LTRs (%)	HTRs (%)
Tacrolimus + MMF	33	33
Tacrolimus + AZA	20	2
Cyclosporine + MMF	13	38
Cyclosporine + AZA	12	4
Cyclosporine/Tacrolimus + SIR	6	7
Tacrolimus	9	4
Cyclosporine	-	3
Other	9	8

Data modified from the registry of the International Society for Heart and Lung Transplantation (ISHLT) (www.ishlt.org/registries).
[a] All of the LTRs and 77 % of the HTRs reported in the ISHLT registry received corticosteroids together with other immunosuppressive drugs.
LTRs, lung transplant recipients; HTRs, heart transplant recipients;
MMF, Mycophenolate mofetil; AZA, Azathioprine; SIR, Sirolimus.

1.3. Infectious complications

Due to the long-term immunosuppressive therapy, transplant recipients are vulnerable to many opportunistic and community-acquired pathogens. Microbes of which the key host defence is mediated by T-lymphocytes are of special importance (e.g. herpesviruses and *Pneumocystis carinii*). Infections are the most common cause of mortality during the first postoperative year after thoracic organ Tx and affect significantly the outcome of the recipients also thereafter (Taylor et al. 2006, Trulock et al. 2006). Furthermore, the infectious complications in LTRs are more common than in any other SOT recipients and occur twice as frequently as in HTRs (Dummer et al. 1986, Kramer et al. 1993, van der Bij and Speich 2003). In addition to immunosuppressive therapy, factors predisposing LTRs to infectious complications include: 1) an allograft exposed continuously to the environment; 2) impaired mucociliary clearance; 3) interrupted lymphatic drainage; 4) denervation of the allograft with diminished cough reflex; 5) damage to bronchial epithelium and 6) burden of microbes from the upper airways, paranasal sinuses, donor lungs or the remaining native lung in single lung Tx (van der Bij and Speich 2003). Over 70 % of the infections in LTRs involve the respiratory tract, and the lungs are the most common site of infection also in HTRs (Maurer et al. 1992, Horvath et al. 1993, Miller et al. 1994).

There exists a typical sequence according to which different microbes cause infections after SOT (Fishman and Rubin 1998). The appearance of the most significant pathogens in HTRs and LTRs is presented in Figure 2. The first month after Tx is influenced predominantly by the infections related to prior surgery and intensive care. The time-period from one to six postoperative months is characterised by a high level of immunosuppression, and, therefore, by the emergence of many opportunistic pathogens. Although the infections beyond six months are increasingly community-acquired, opportunistic pathogens are still detected especially when increased epidemiologic

exposure, an augmented level of immunosuppression or discontinuation of former prophylaxis are present (Villacian and Paya 1999, Speich and van der Bij 2001).

Bacterial pneumonia is the most common infection after thoracic organ Tx and accounts for the majority of infection-related deaths in LTRs (Miller et al. 1994, Kramer et al. 1993). The most common causative bacteria in LTRs include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, enterobacteriaceae, *Enterococcus* sp., and *Haemophilus influenzae* (Kramer et al. 1993, Chan et al. 1996). Other significant bacterial infections include septichaemia related to vascular catheters and wound infection in the immediate postoperative period.

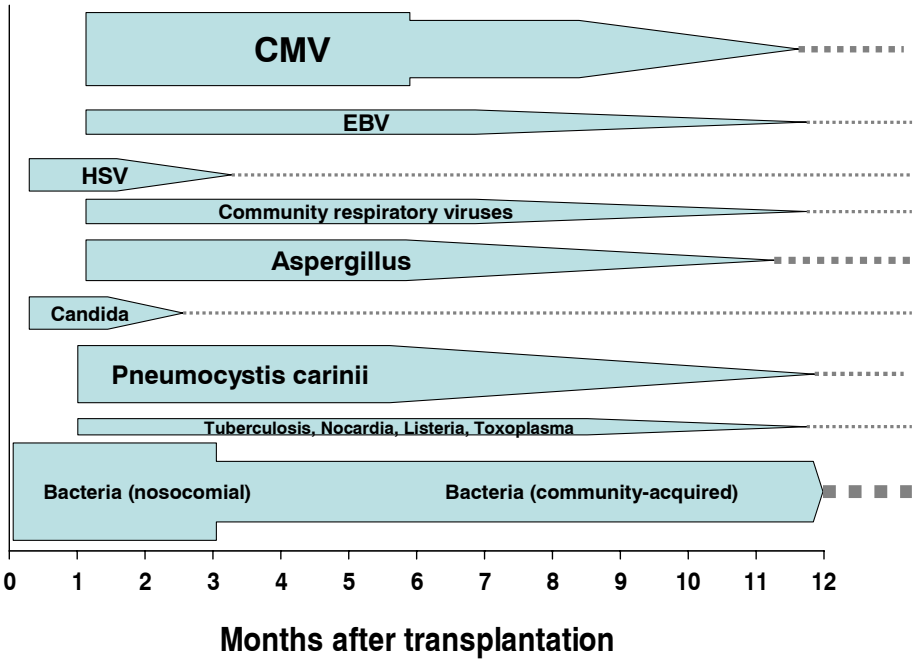

Figure 2. Appearance of the most significant infections after thoracic organ transplantation. Bars indicate the common period for the onset of infection. Dotted lines show the continued risk of infection. Weight of the bars and lines indicate the significance of infection during different time periods. CMV, cytomegalovirus; EBV, Ebstain-Barr virus; HSV, Herpes simplex virus.

The major importance of CMV after LTx and HTx is discussed in Section 3. Herpes simplex virus (HSV) may cause serious infections in HTR or LTR, but prophylaxis with acyclovir or ganciclovir is effective in preventing HSV (Smyth et al. 1990). The clinical significance of Ebstain-Barr virus (EBV) in transplant recipients is primarily related to its involvement in the development of the post-transplant lymphoproliferative disorder (PTLD) (Gray et al. 1995, Paya et al. 1999). Although the overall role of respiratory viruses, such as respiratory syncytial virus (RSV), influenza, parainfluenza, and adenovirus, in transplant recipients is not known, they may cause serious lower respiratory tract infection in LTRs (Palmer et al. 1998a).

Invasive aspergillosis (usually caused by *A. fumigatus*) is a life-threatening infection with a mortality rate of over 60 % among SOT recipients (Singh 2000). LTRs are extremely prone to this complication with reported incidences of 12 - 16 % (Yeldandi et al. 1995, Mehrad et al. 2001 Nunley et al. 2002). Therefore, prophylactic or pre-emptive antifungal therapy is commonly used during the first postoperative months after LTx (Dummer et al. 2004). *Candida* species are responsible for many serious infections (e.g. sepsis) in transplant recipients, but involvement of the lung is rare with the exception of anastomotic infections reported in LTRs (Palmer et al. 1998b).

Before the era of chemoprophylaxis, pneumonia caused by *P. carinii* (nowadays named as *Pneumocystis jiroveci*) was reported even in up to 40 and 88 % of the HTRs and LTRs, respectively (Gryzan et al. 1988, Olsen et al. 1993). The widely used and highly effective chemoprophylaxis has significantly decreased the role of this organism, but cases of *P. carinii* pneumonia (PCP) are reported after discontinuation of the prophylaxis and rarely also during the chemoprophylaxis (Gordon et al. 1999, Faul et al. 1999).

1.4. Noninfectious complications

Acute graft failure is the most common cause of death during the immediate postoperative period, after which acute rejection and infection are the most important complications during the first year after LTx and HTx. The diagnosis of acute rejection in LTRs is characterized by perivascular mononuclear infiltrates with or without accompanying lymphocytic bronchitis or bronchiolitis. Acute rejection is graded by the ISHLT Lung Rejection Study Group from minimal A1 (infrequent perivascular infiltrates) to severe A4 (diffuse perivascular, interstitial, and alveolar infiltrates). Similarly, the lymphocytic bronchitis is graded from minimal B1 (rare mononuclear cells in the submucosa) to severe B4 (dense infiltrate of mononuclear cells with dissociation of epithelium from the basement membrane) (Yousem et al. 1996). The definitions and grading system for acute rejection in HTRs has also been published under the direction of the ISHLT (Stewart et al. 2005).

The major cause of late graft failure and the main limitation to the long-term survival of the recipients is chronic allograft dysfunction (Taylor et al. 2006 Trulock et al. 2006). Its manifestation is cardiac allograft vasculopathy (CAV) in HTRs and bronchiolitis obliterans syndrome (BOS) in LTRs. BOS is defined as persistent airflow obstruction demonstrated by spirometry in the absence of other conditions affecting the graft function (Estenne et al. 2002). Typical findings (e.g. air trapping) on high-resolution computed tomography (HRCT) supports the diagnosis of BOS (Bankier et al. 2001, Estenne et al. 2002). Obliterative bronchiolitis (OB) is the histological diagnosis of the condition. It is defined as peribronchial inflammation and obliteration of small and medium-sized bronchioli (Yousem et al. 1996). Characteristic features of CAV are intimal thickening and stenosis of the minor and major coronary arteries, which is clinically demonstrated by coronary angiography or intravascular ultrasound (Billingham 1992, Yeung et al. 1995, Costanzo et al. 1998). Approximately 50-60 % of the LTRs suffer from BOS and one third of the

HTRs from CAV five years after transplantation (Boehler et al. 2003, Taylor et al. 2006). Although acute rejection is the best-documented risk factor for BOS and CAV, also infections (e.g. viruses) are associated with the development of chronic allograft injury (Sharples et al. 2002, Valantine 2004, Taylor et al. 2006). BOS, in turn, pre-disposes the lung allograft to recurrent and resistant infections (e.g. Pseudomonas bronchitis and pneumonia) (Kramer et al. 1993, Reichenspurner et al. 1996, van der Bij and Speich 2003). Long-term immunosuppressive medication also places transplant recipients at risk of cancer, especially lymphomas (PTLD) and skin malignancies. The most common causes of death in different time-intervals after LTx and HTx are shown in Figure 3.

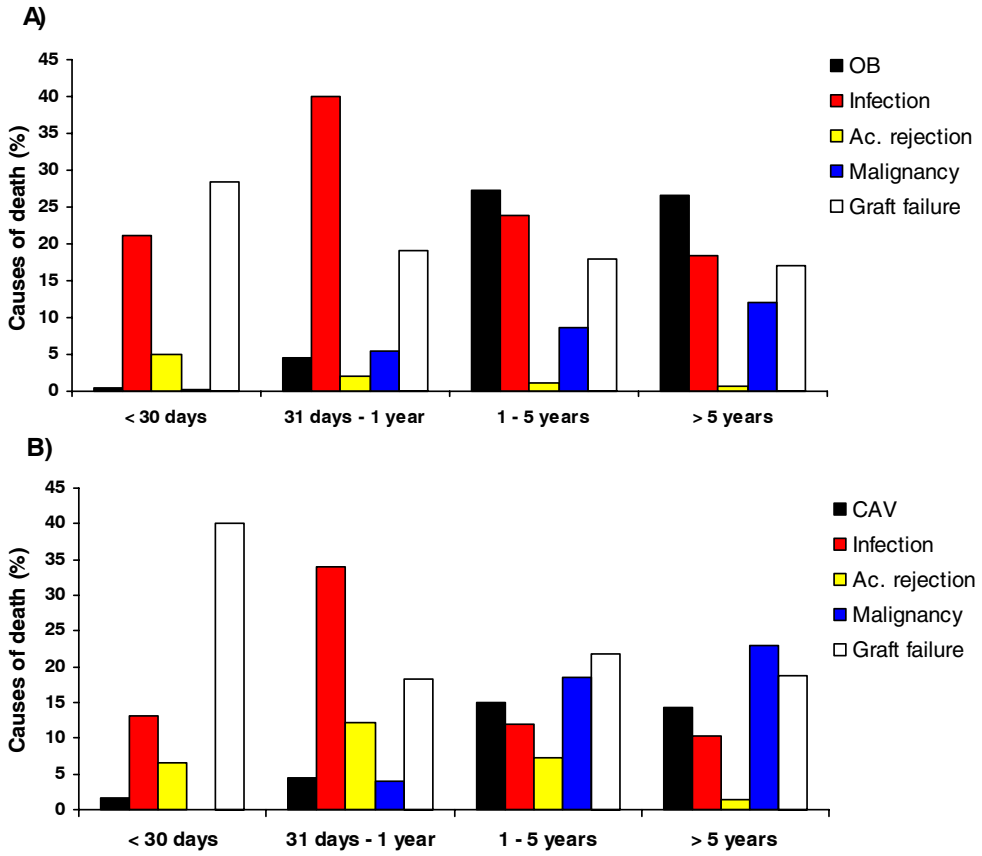

Figure 3. The most common causes of death after lung (A) and heart (B) transplantation.
Data modified from the registry of ISHLT (www.ishlt.org/registries).
OB, obliterative bronchiolitis; CAV, cardiac allograft vasculopathy. NOTE: Most of the graft failures occurring after the first year after transplantation are highly suggestive of being due to OB or CAV.

2. Bronchoscopy in transplant recipients

The aetiology of pneumonia in transplant recipients differs from that in the general population, and the manifestations of respiratory infections are variably modified by the immunosuppressive therapy. Respiratory symptoms, fever, and radiographic infiltrates may also be due to non-infectious complications such as malignancy, pulmonary oedema, anastomotic complications (LTRs) or acute rejection (LTRs). Thus, the definite diagnosis is of major importance in choosing adequate therapy for a transplant recipient with a suspected respiratory infection, and bronchoscopy is widely recommended as the initial invasive procedure in this clinical context (Nusair and Kramer 1999, Speich and van der Bij 2001).

2.1. Bronchoscopic techniques

Bronchoscopy allows visualisation of the whole tracheobronchial tree providing a method to obtain samples from the lower respiratory tract. In order to receive samples without contamination from the upper airway secretions bronchial brushings with a protected specimen brush (PSB) may be used. The brush is inside a special single lumen or double lumen catheter when passed through the working channel of the flexible bronchoscope into a small bronchus where the actual brushing takes place (Wimberley et al. 1979).

Bronchoalveolar lavage (BAL) is the most commonly used method to receive samples for microbiological studies. The bronchoscope is wedged to a small segmental or subsegmental bronchus, a fairly large volume (usually 100-240 ml) of saline is installed, and the BAL fluid (BALF) representing specimen from the alveolarlevel is retrieved by low pressure suction (Baughman et al. 1994, Taskinen et al. 1994).

Transbronchial lung biopsy (TBB) is a method to receive samples of lung parenchyma. In this technique, the bronchoscope is placed in a subsegmental bronchus and a biopsy forceps is passed forward into the periphery of the lung near the pleura in order to get a samle containing alveoli (Ioanas et al. 2001). Brushing, BAL, and TBBs are performed in the area with the greatest radiologic or visual abnormality. In addition to these techniques, endobronchial biopsies and bronchial washings from intrabronchial lesions are easily performed during the bronchoscopy.

2.2. Microbes demonstrated in bronchoscopic specimens

The clinical significance of various microbes detected in bronchoscopic specimens depends on the organ transplantated (LTx or other SOT), the microbiological methods used for demonstration of the pathogen, and other findings supporting the infection to be caused by the organism (e.g. radiographic appearance).

Bacteria are the most commonly detected pathogens in BALF, but the bacterial findings have to be interpreted with caution. The mouth and upper airways are colonized with bacteria, and the differentiation between contamination and causative pathogens may be difficult. Quantitative bacterial cultures and the PSB technique have been developed to resolve this problem (Wimberley et al. 1979, Ioanas et al. 2001). Some bacteria, such as *Legionella*, Nocardia and M. tuberculosis, are always pathogens when found in respiratory specimens.

The demonstration of *Aspergillus* in BALF (Fig. 4a) or PSB samples together with compatible clinical and radiographic features is suggestive of invasive aspergillosis, though histological confirmation of the diagnosis by biopsy or transthoracic needle aspiration is needed (Nicod et al. 2001, Ascioglu et al. 2002, van der Bij and Speich 2003). Characteristic aspergillus

tracheobronchitis may also be detected during the bronchoscopy. Sole *Aspergillus* colonization in LTRs is associated with anastomotic complications and the development of invasive aspergillosis and, therefore, anti-fungal therapy is frequently initiated if *Aspergillus* is detected in bronchoscopic samples (Cahill et al. 1997, Nathan et al. 2000, Nunley et al. 2002, Dummer et al. 2004). *Candida* sp. (mainly *C. albicans*) is frequently detected in BALF and PSB samples, but it usually represents a contamination from the upper airways without any major diagnostic significance (Rello et al. 1998).

P. carinii (Fig. 4b) is an intra-alveolar organism and is easily detected in BALF. Thus, BAL is the procedure of choice in establishing PCP with the diagnostic yield reaching up to 90 % (Schulmann et al. 1988, Baughman et al. 1994). TBBs taken together with BAL may further enhance the yield of bronchoscopy, but it is associated with an increased risk of complications (Baughman et al. 1994).

BAL is generally found to be sensitive in detecting CMV in the lung, but methods for the demonstration of the virus as well as the definition of CMV pneumonia slightly differ between the studies (Schulman et al. 1991, Stenberg et al. 1993, Baz et al. 1996, Torres et al. 2000, Hopkins et al. 2002). Characteristic viral inclusions in BALF (Fig. 4 c) or demonstration of the virus in lung tissue (TBB) by immunohistochemistry, *in situ* hybridisation or viral inclusions are widely accepted as confirmation of CMV pneumonia (Ljungman et al. 2002a, Kotloff et al. 2004). The demonstration of CMV by viral culture, antigen detection or a DNA/RNA-based assay in BALF has been presented to allow presumptive diagnosis of CMV pneumonia when the clinical findings support the diagnosis (Trulock 1999, Preiksaitis et al. 2005). However, positive CMV cultures from BALF may reflect viral shedding into the respiratory tract without a major clinical significance (Ruutu et al. 1990, Mann et al. 1997). Similarly, detecting CMV DNA in BALF by the qualitative polymerase chain reaction (PCR) alone is considered insufficient in the diagnosis of CMV

pneumonia, but quantitative PCR has been found useful in recent studies (Ljungman et al. 2002a, Westall et al. 2004, Chemaly et al. 2004, Chemaly et al. 2005). The concomitant detection of CMV in BALF and blood is suggested to strengthen the evidence of CMV pneumonia (Preiksaitis et al. 2005).

Community-acquired respiratory viruses can be detected in BALF by antigen detection, PCR or culture (Weinberg et al. 2002). Although these viruses in BALF may reflect upper airway infection, they are able to cause pneumonia in SOT recipients and, therefore, should not be ignored (Palmer et al. 1998a, Speich and van der Bij 2001). Although there is no standard method to detect HHV-6 and HHV-7 in BALF, PCR-based methods have been used (Ross et al. 2001, Jacobs et al. 2003, Neurohr et al. 2005).

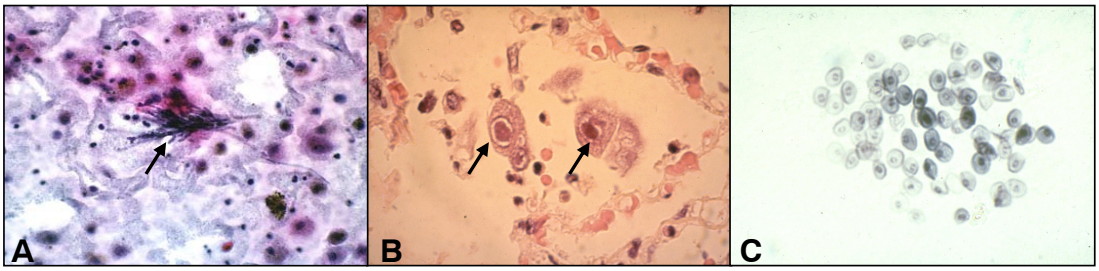

Figure 4. Characteristic findings in bronchoalveolar lavage fluid. A, Aspergillus hyphae; B, CMV viral inclusions in alveolar macrophages; C, pneumocysts in giemsa silver-methenamine stain. Figures provided from the Transplantation laboratory, University of Helsinki and Helsinki University Central Hospital.

2.3. Bronchoscopy in lung transplant recipients

Bronchoscopy plays a central role in the management of LTRs. As in other SOT recipients, bronchoscopy is useful in the diagnosis of infection, but the procedure is also irreplaceable for the detection of acute rejection and airway complications in LTRs (Trulock 1997). The standard method for the diagnosis of acute rejection in the lung allograft is TBB with a sensitivity of 70 - 90 % and specificity of 90 - 100 % (Scott et al. 1991, Trulock et al. 1992, Trulock 1997). At least five specimens containing lung parenchyma are needed to achieve an acceptable diagnostic yield. Although complications involving the bronchial anastomosis (e.g. dehiscence, stenosis, and bronchomalasia) have decreased since the early days of LTx, they still occur in some recipients (Ruttmann et al. 2005). Bronchoscopy is the key diagnostic procedure for these problems, and therapeutic interventions including balloon dilatation, laser recanalization or bronchial stent placement can also be performed through the bronchoscope (Higgins et al. 1994).

The overall yield of bronchoscopy performed on LTRs in different studies is presented in Table 3. When performed on LTRs with clinical symptoms or findings referring to acute rejection or infection, bronchoscopy is a well-established diagnostic procedure with a considerably high yield (Chan et al. 1996, Hopkins et al. 2002). In addition, regularly scheduled bronchoscopies are performed on asymptomatic LTRs in order to detect clinically silent rejection or infection (Kukafka et al. 1997). Some authors have found a reasonably good diagnostic yield of about 50-60 % from these surveillance bronchoscopies (Table 3). In contrast, other studies have reported much lower yields, and routine bronchoscopies are not proven to decrease mortality or the development of BOS (Tamm et al. 1997, Valentine et al. 2002).

Table 3. The diagnostic yield of bronchoscopy in lung transplant recipients. The criteria for significant diagnoses received by bronchoscopy influence the reported yields.

Study	Clinically indicated FBs		Surveillance FBs		Specimens
	N	Yield (%)	N	Yield (%)	studied
Trulock et al. 1992	88	69	90	57	TBB
Sibley et al. 1993	128	66	133	43	TBB
Guilinger et al. 1995	-	-	355	25	TBB+BAL
Chan et al. 1996	282	67	39	58	TBB+BAL
Baz et al. 1996	69	48	157	26	TBB+BAL
Kesten et al. 1996[a]	-	-	102	10	TBB
Hopkins et al. 2002	344	86	836	19	TBB
Chakinala et al. 2004	-	-	629	34	TBB

FB, Flexible broncoscopy; TBB, Transbronchial biopsy; BAL, Bronchoalveolar lavage.
[a] Only TBBs performed beyond two years after transplantation are included.

2.4. Bronchoscopy in other solid organ transplant recipients

The major indication for bronchoscopy in transplant recipients is suspected respiratory infection. The diagnostic yield of bronchoscopy in different SOT recipients is summarized in Table 4. The differences in the organ transplantated, antimicrobial and immunosuppressive medication given to the recipient, the bronchoscopic samples achieved, and the criteria for the microbiological diagnosis of pneumonia explain the variation between the studies. Nevertheless, the diagnoses established by bronchoscopy have been of considerable clinical significance in terms of leading to a change in the medical therapy in about 30 - 40 % of recipients (Torres et al. 2000, Sternberg et al. 1993). The most common causative microbes in SOT recipients have included bacteria, CMV, and *P. carinii* (Torres et al. 2000, Reichenberger et al. 2001).

Table 4. The diagnostic yield of bronchoscopy in solid organ transplant recipients with suspected respiratory infection.

Organ transplanted	FBs (n)	Yield of FB (%)	Specimens			Reference
			BAL(n)	PSB(n)	TBB(n)	
Heart	39	62	+ (35)	-	+ (37)	Schulman et al. 1988 [a]
Kidney	58	55	+ (58)	-	-	Sternberg et al. 1993
Kidney	33	61	+ (33)	-	+ (33)	Cazzadori et al. 1995
Kidney	91	69	+ (91)	-	-	Reichenberger et al. 2001
Kidney	64	59	+ (NA)	+ (NA)	+ (NA)	Chang et al. 2004
Liver	60	48	+ (58)	+ (60)	-	Torres et al. 2000

FB, Flexible broncoscopy; BAL, bronchoalveolar lavage; PSB, protected specimen brush; TBB, transbronchial biopsy
NA, the proportion of bronchoscopies with each technique is not reported;
[a] Bronchial brushings were performed, but the technique was not specified.

2.5. Complications of the bronchoscopy

Bronchoscopy is considered a safe procedure in transplant recipients. However, major adverse events such as bleeding, pneumothorax, respiratory insufficiency, and extremely uncommon cases of fatality have been reported (Hopkins et al. 2002, Chhajed et al. 2003, Dransfield et al. 2004). The overall complication rate for bronchoscopy after LTx is reported to be approximately 2 - 9 % (Trulock et al. 1992, Hopkins et al. 2002, Dransfield et al. 2004). Although the complications related to bronchoscopy are not widely reported in SOT recipients other than LTRs, some major adverse events (cardiac arrhythmia, hypotension, and pneumothorax) were detected in HTRs by Schulman and co-workers (Schulman et al. 1988). The frequency of complications in mixed populations of immunocompromised patients has ranged from 2 to 21 % (Cazzadori et al. 1995, Rano et al. 2001, Jain et al. 2004). This relatively large variation in the reported complication rates of bronchoscopy is mainly due to different definitions for complications (e.g. the amount of bleeding regarded as “complication”) as well as to the patients studied.

3. Cytomegalovirus (CMV) infection in lung and heart transplant recipients

3.1. CMV

Until today, eight herpes viruses have been isolated in man: HSV-1, HSV-2, CMV, varicella zoster virus (VZV), EBV, HHV-6, HHV-7, and HHV-8. CMV is a ubiquitous member of the herpes virus family with a world-wide seroprevalence of 50-100 % depending on the population studied (Sissons et al. 2002a, Alanen et al. 2005). In immunocompetent individuals, CMV infection usually manifests as a mild or asymptomatic infection during the first two decades of life. After primary infection, CMV is maintained in the host as latent infection controlled by the normally functioning immune system. Cytotoxic T lymphocytes and natural killer cells are probably the most important part of the defense mechanism against CMV, while humoral immunity may be of less importance (Harari et al. 2004). The main reservoir of latent CMV is thought to be blood mononuclear leukocytes and haematopoietic progenitor cells, though CMV latency is suggested also in other cell types (e.g. endothelial cells) (Taylor-Wiedeman et al. 1991, Kondo et al. 1994, Sissons et al. 2002b). The immunosuppressive therapy, alloimmune responses, and release of proinflammatory cytokines place transplant recipients at risk of CMV reactivation from latency, amplification of the viral replication during active CMV infection, and development of tissue invasive CMV disease (Rubin 2001, Rowshani et al. 2005). Therefore, CMV remains as the most important single pathogen in transplant recipients causing significant morbidity and mortality (Rubin 2001).

3.2. Definition and diagnosis of CMV infection

In SOT recipients, *CMV infection* is defined as isolation of the virus or detection of viral proteins or nucleic acids in any body fluid (usually blood) or tissue specimen (Ljungman et al. 2002a). The *CMV disease* is confirmed by histological evidence of tissue invasion by the virus in the organ involved (Ljungman et al. 2002a, Zamora 2002). In addition, a characteristic syndrome after exclusion of other causes in the presence of CMV infection is considered to allow the presumptive diagnosis of the CMV disease (Zamora 2005, Preiksaitis et al. 2005). Several methods are currently available for the demonstration of CMV infection in transplant recipients.

Culture. The recovery of CMV by culture has been a traditional method for the diagnosis of CMV infection. About 20 years ago the rapid shell vial assay using antibodies directed to CMV early antigens was developed to decrease the time needed for CMV culture (Gleaves et al. 1984, Gleaves et al. 1985). However, CMV cultures from blood are time-consuming and insensitive compared to CMV assays detecting pp65antigen or viral nucleic acids (Weinberg et al. 2000, van der Bij and Speich 2001).

Antigenemia assay. The antigenemia assay is based on the detection of CMV antigen in peripheral blood leukocytes (antigenemia) by direct immunostaining using monoclonal antibodies against the CMV lower matrix phosphoprotein pp65 (van der Bij et al. 1988, The et. al. 1995). Quantitative results are expressed as the pp65-positive polymorphonuclear leukocytes (PMNL) per number of cells evaluated. Although some conflicting data exist, the antigenemia test is reliable in detecting CMV infection and predicting CMV disease (Egan et al. 1995, Kelly et. al. 2000, Weinberg et al. 2000, Gerna et al. 2003). The need for immediate processing of samples, the variety of in-house modifications of the method, and the subjective nature of quantification are the main limitations of

the antigenemia test in clinical practice (Razonable et al. 2002). To resolve these difficulties, molecular assays to detect CMV DNA or RNA have been developed.

DNAemia assays. The assays using PCR-based methods to detect CMV DNA in blood (DNAemia) are increasingly recognized as rapid and useful in demonstrating CMV infection. Standardized and quantitative PCR assays using serum, plasma or peripheral blood leukocytes are commercially available. The hybrid capture assay is a non-PCR-based method to detect CMV DNAemia, but it has not been studied as widely as the PCR-based assays (Mazulli et al. 1999, Bhorade et al. 2001). A good correlation between the DNAemia and antigenemia levels has been detected in SOT recipients (Pang et al. 2003, Piiparinen et al. 2004). High CMV DNAemia has also been shown to predict and correlate to the CMV disease in most reports, though it was questioned in two recent studies (Rollag et al. 2002, Caliendo et al. 2002, Humar et al. 2004, Chemaly et al. 2004).

RNAemia assays. The most commonly used method to detect CMV RNA in blood (RNAemia) is to demonstrate CMV late pp67 message RNA (mRNA) by nucleic acid sequence-based amplification (NASBA) (Blok et al. 1998). The presence of pp67-mRNAemia indicates active viral replication (Razonable 2002). Nevertheless, the assay is qualitative and has been found to be less sensitive than the DNAemia and antigenemia tests in most, but not all, previous studies (Gerna et al. 1999, Oldenburg et al. 2000, Blok et al. 2000, Gerna et al. 2003).

Demonstration of CMV in tissue specimens. Although the criteria differ from organ to organ, the diagnosis of the CMV disease should be confirmed in tissue specimens by virus isolation, histopathologic features, immunohistochemistry, or in situ hybridization (Ljungman et al. 2002a).

3.3. Risk factors and impact of CMV infection

The CMV serostatus of the recipient (R) and donor (D) is the most important risk factor for the development and severity of CMV infection. Recipients with no previous history of CMV infection receiving a graft from a seropositive donor (R-/D+) are at high risk of primary infection which commonly manifests as severe disease, since no previous host defences against the virus are present (Wreghitt et al. 1989, Duncan et al. 1991). Without effective chemoprophylaxis a symptomatic CMV disease has been reported to occur in up to 91 % of the thoracic organ recipients with a D+/R- match, while the incidence in seropositive (R+) LTRs and HTRs ranges from 30 to 68 % and from 17 to 46 %, respectively (Duncan et al. 1991, Merigan et al. 1992, Ettinger et al. 1993, Koskinen et al. 1993, Grossi et al. 1995, Camargo et al. 2001). When asymptomatic activation of the virus is included and no antiviral prophylaxis is used, CMV infection has been reported in up to 81 - 100 % and 51 - 93 % of the LTRs and HTRs at risk (D+ or R+), respectively (Duncan et al. 1991, Ettinger et al. 1993, Grossi et al. 1995, Soghikian et al. 1996, Camargo et al. 2001). In contrast, CMV infections in adult recipients with a D-/R- match are rare. It is difficult, however, to determine the exact incidences, since the risk factors and the definition for CMV infection differ between the studies. The risk of CMV infection is also dependent on the intensity of immunosuppression, especially the use of anti-lymphocyte antibodies, as well as on the organ transplanted (Jamil et al. 2000, Zamora 2004a). The frequency and severity of CMV infections are higher in LTRs than in any other SOT recipients, probably due to the relatively intensive immunosuppressive therapy and the lung allograft carrying large amounts of the virus (Balthesen et al. 1993). Thus, some authors consider all LTRs (excluding those with an R-/D- match) to be at high risk of CMV infection (Zamora 2004a).

The harmful consequences of CMV infection in transplant recipients can be divided into direct mortality and morbidity of the clinical disease and indirect effects of the virus leading to acute and chronic allograft injury (Zamora 2004a). Clinical manifestations of the CMV disease include fever, leukopenia, trombocytopenia, pneumonia, hepatitis, encephalitis, myocarditis, retinitis, and gastrointestinal disease (Rubin and Fishman 1998, Ljungman et al. 2002a). In addition, asymptomatic CMV infection (e.g. antigenemia or DNAemia) is frequently detected in LTRs and HTRs (Gerna et al. 2003). An association between CMV infection and the development of chronic allograft injury (BOS and CAV) has been increasingly found, though this relationship is debated in some of the studies (Valantine et al. 1999, Westall et al. 2003, Potena et al. 2003, Tamm et al. 2004, Ruttmann et al. 2006). CMV infection is also suggested to increase the frequency and severity of the acute rejection episodes which, in turn, are risk factors for BOS and CAV (Sharples et al. 2002, Zamora 2004a, Taylor et al. 2006, Potena et al. 2006). The bidirectional relationship between CMV and allograft injury emerges from the perception that active CMV infection promotes acute rejection by inducing the production of inflammatory mediators, and the acute alloimmune response, in turn, activates latent CMV infection. Furthermore, the CMV infection-enhanced immune activation and smooth muscle cell (SMC) proliferation may lead to the occlusion of blood vessels and bronchioles and thereby to BOS and CAV (Lemström et al. 1993, Lemström et al. 1994, Tikkanen et al. 2001, Zamora 2004a, Lemström et al. 2005). Through its suppressive effects on host defences CMV has been shown to increase susceptibility to opportunistic infectious agents, such as fungi (Yeldandi et al. 1995). CMV may also work synergistically with other pathogens to cause the disease (e.g. PTLD) (Pescovitz 2006). The effects of CMV after LTx and HTx are summarized in Figure 5.

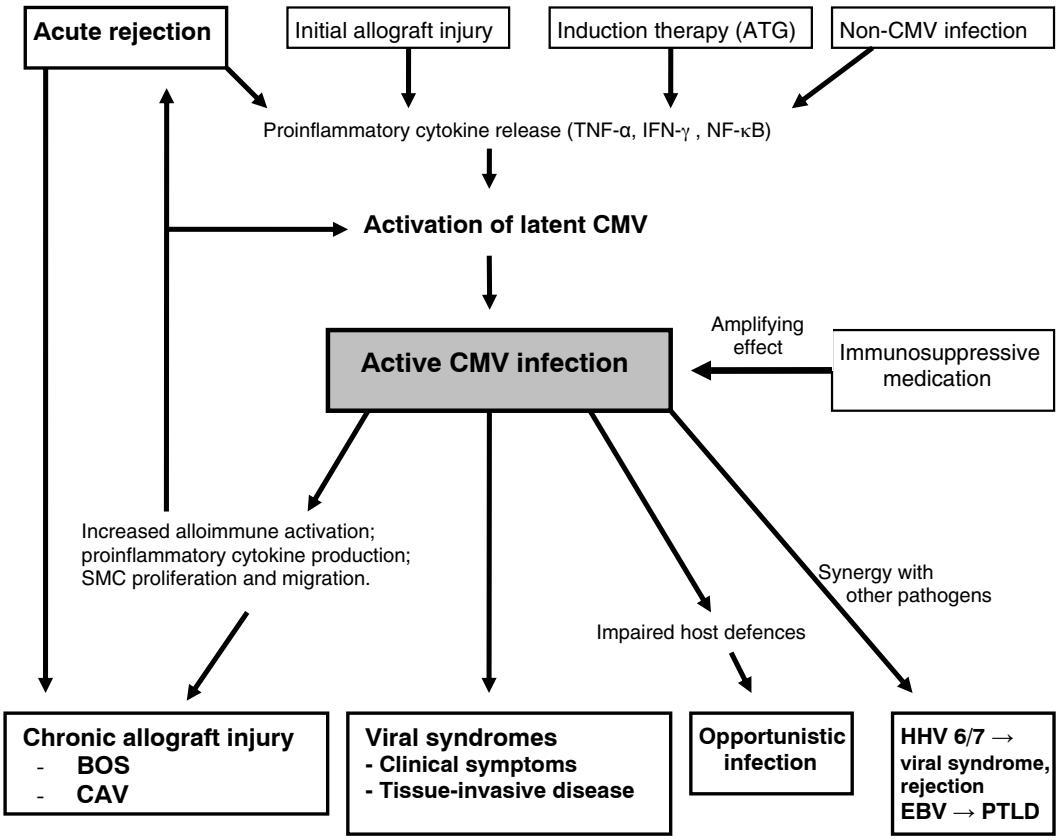

Figure 5. The effects of cytomegalovirus (CMV) infection in lung and heart transplant recipients.
ATG, antithymocyte globulin; TNF-α, Tumour necrosis factor α; NF-κB, nuclear factor-κB; IFN-γ, interferon-γ; SMC, smooth muscle cell; BOS, bronchiolitis obliterans syndrome; CAV, cardiac allograft vasculopathy; EBV, Ebstain-Barr virus; PTLD, post-transplant lymphoproliferative disorder; HHV 6/7, Human herpesvirus 6/7.

3.4. Prevention and treatment of CMV infection

To prevent and treat CMV infections, antiviral agents may be administered to all recipients considered to be at risk of CMV infection (prophylaxis), when a positive laboratory test or a certain cut-off value of a quantitative assay is detected (pre-emptive therapy) or when a symptomatic CMV infection occurs (rescue therapy) (van der Bij and Speich 2001). The drug of choice for the therapy and prophylaxis against CMV infection is ganciclovir. Its prodrug valganciclovir has recently been shown effective in the pre-emptive therapy against CMV infection after SOT (Devyatko et al. 2004, Diaz-Pedroche et al. 2006). The advantage of valganciclovir is its high bioavailability in oral administration (Wiltshire et al. 2005).

Prophylaxis with intravenous ganciclovir or oral valganciclovir is effective in preventing CMV infection and disease in LTRs and HTRs (Merigan et al. 1992, Duncan et al. 1992, Soghikian et al. 1996, Humar et al. 2005). Chemoprophylaxis against CMV may also reduce the risk of BOS and CAV (Potena et al. 2006, Ruttmann et al. 2006). Furthermore, CMV prophylaxis decreased the all-cause mortality during the first postoperative year after SOT in two recent meta-analyses, but studies on LTRs were not included (Hodson et al. 2005, Kalil et al. 2005). Universal chemoprophylaxis against CMV is recommended to all LTRs at risk (D+ or R+) and to HTRs with a D+/R- match, but the optimal regimen and duration of prophylaxis are unclear (Rubin 2000, Zamora et al. 2005). Nevertheless, CMV infections occur after cessation of the prophylaxis necessitating the surveillance and treatment of CMV infection also when anti-CMV prophylaxis is initially used (Duncan et al. 1994, Soghikian et al. 1996, Humar et al. 2005, Potena et al. 2006, Ruttmann et al. 2006).

The strategy of the pre-emptive therapy is based on the detection of CMV infection (e.g. antigenemia, DNAemia or RNAemia) and the institution of an antiviral therapy before a full-blown CMV disease develops. The pre-emptive therapy needs to be guided by a convenient, reliable, and timely diagnostic surveillance test which will identify CMV infection quickly enough to prevent the CMV disease to develop. Traditionally, the CMV pp65 antigenemia test has been used for surveillance of CMV infection and has proved reliable in guiding the pre-emptive therapy (Egan et al. 1998, Kelly et al. 2000). Assays detecting CMV DNAemia by PCR are recommended as good alternatives to the antigenemia test in guiding the pre-emptive therapy (Rubin 2000, Zamora 2005, Preiksaitis et al. 2005). However, their overall usefulness is not widely studied in HTRs and LTRs. Although in some studies the pp67 mRNAemia test has been relatively insensitive, Gerna and coworkers regarded this assay as an efficient method in the guidance of the pre-emptive therapy (Blok et al. 2000, Gerna et al. 2000, Gerna et al. 2003).

While modern treatment and prophylaxis strategies have undoubtedly declined the mortality and morbidity associated with CMV infections, the optimal tests and relevant thresholds for guidance of the antiviral therapy in LTRs and HTRs still remain to be determined.

4. Human herpesvirus(HHV)-6 and HHV-7 in transplant recipients

4.1. HHV-6 and HHV-7

HHV-6 and HHV-7, together with their close relative CMV, form the beta-herpesvirus family (Black 1999, Dockrell 2003). HHV-6 is a lymphotropic virus which uses a CD46 molecule as receptor and may also infect other cell types, such as monocytes or even epithelial and endothelial cells (Dockrell and Paya 2001, De Bolle et al. 2005). HHV-7 uses a CD4 molecule as receptor and is more selectively lymphotropic (Black 1999). The primary HHV-6 and -7 infection manifests as *exanthema subitum* and roseola during early childhood (Yamanishi et al. 1988, Black 1999). As the seroprevalence of HHV-6 and -7 in the adult population is over 90 %, primary infections in adult individuals are rare (Levy et al. 1990, Clark et al. 1993, Humar 2006).

4.2. Impact of HHV-6 and HHV-7

After primary infection, HHV-6 and HHV-7 can establish a latent infection for lifetime and be reactivated during immunosuppression. Reactivation of HHV-6 and, to a lesser extent, of HHV-7 has been reported to be relatively common after solid-organ and stem cell Tx (Griffiths et al. 1999, Lautenschlager et al. 2000, Mendez et al. 2001, Emery 2001, Yoshikawa 2004, Volin et al. 2004). However, most of the studies after SOT have evaluated liver transplant recipients, and only three studies have included LTRs. Jacobs and coworkers detected HHV-6 in blood by PCR or culture in 66 % of the LTRs, and Neurohr et al. found HHV-6 DNA from the BALF in 31 % of the patients (Jacobs et al. 2003, Neurohr et al. 2005). In contrast, Michaelides et al. did not detect HHV-6-DNAemia in any of the 26 LTRs studied (Michaelides et al. 2002). Until today, no prospective studies on HHV-7 after LTx are available.

There is no standard assay for the detection of HHV-6 and -7. Most of the studies have employed PCR-based methods, but the detection of viral DNA in peripheral blood or in leukocytes is also possible in the case of latent infection (Ljungman 2002b, Razonable et al. 2005). Quantitative PCR methods have been developed to resolve this problem, but clinically relevant threshold levels for HHV-6 or -7 DNAemia are not known (Yoshikawa 2004). The demonstration of HHV-6- and HHV-7-specific antigens in peripheral blood mononuclear cells (PBMCs) provides an alternative method to detect active viral replication (Lautenschlager et al. 2000).

In transplant recipients most of the HHV-6 and HHV-7 activations are asymptomatic (Jacobs et al. 2003, Razonable et al. 2005, Humar 2006). Nevertheless, HHV-6 has been associated with multiple clinical manifestations (skin rashes, hepatitis, bone marrow suppression, interstitial pneumonitis, and encephalitis), and even life-threatening infections have been reported (Singh et al. 1997, Zerr et al. 2002, Volin et al. 2004, De Bolle et al. 2005). The causal relationship between the symptoms and HHV-6 is, however, often uncertain, and other infections occurring concomitantly with HHV-6 may be responsible for the clinical manifestations. Although an increasing number of studies evaluating also HHV-7 in SOT recipients exists, the clinical significance of this virus is still poorly understood (Griffiths et al. 1999, Razonable et al. 2003, Razonable et al. 2005).

HHV-6 activation has been associated with allograft rejection and dysfunction in liver transplant recipients (Griffiths et al. 1999, Lautenschlager et al. 2000, Humar et al. 2002). In a recent study on LTRs, the demonstration of HHV-6 in BALF also increased the risk of BOS (Neurohr et al. 2005). Furthermore, the concomitant appearance of HHV-6 or HHV-7 together with CMV is frequently detected and may lead to a more severe viral infection suggesting interactions between the three beta-herpesviruses (DesJardin 2001, Humar et al. 2002, Lautenschlager et al. 2002, Humar 2006). These findings raise the possibility that all three beta-herpesviruses may be involved in the indirect

effects originally suggested only to be due to CMV and the manifestations of the CMV disease may be partly related to HHV-6 or -7.

4.3. Prevention and treatment of HHV-6 and HHV-7

Ganciclovir, foscarnet, and cidofovir have in vitro activity against HHV-6 and HHV-7 (Ljungman 2002b, De Clercq et al. 2001). Although there are several case reports suggesting clinical response on HHV-6-related disease by ganciclovir or foscarnet therapy, no controlled studies assessing the efficacy of antiviral agents in the treatment of HHV-6 or -7 have been performed (Mookerjee and Vogelsang 1997, Zerr et al. 2002, Ljungman 2002b). Antiviral prophylaxis with ganciclovir may decrease or delay the appearance of HHV-6, but compelling data on the usefulness of antiviral prophylaxis against HHV-6 and -7 is lacking (Razonable et al. 2005, Galarraga et al. 2005). No controlled studies evaluating the efficacy of the preventive antiviral strategies on HHV-6 or -7 in LTRs exist.

AIMS OF THE STUDY

The overall purpose of this study was to expand the knowledge in the infectious complications after lung and heart transplantation with a special emphasis on the diagnostic usefulness of bronchoscopy and the detection of CMV, HHV-6, and HHV-7 infections.

The specific aims of the study were the following:

1) to evaluate the diagnostic yield, clinical impact, and safety of the bronchoscopy in HTRs with a suspected respiratory infection;

2) to evaluate the diagnostic yield, clinical impact, and safety of bronchoscopy in the diagnosis and surveillance of respiratory infections and other postoperative complications in LTRs;

3) to compare the CMV antigenemia, DNAemia, and mRNAemia tests in detecting CMV infection in thoracic organ transplant recipients with a special emphasis on the feasibility of the DNAemia and mRNAemia assays in guiding the pre-emptive therapy;

4) to determine the incidence of HHV-6 and HHV-7 antigenemia, evaluate their association with clinical symptoms, and describe the effect of the antiviral prophylaxis against CMV, HHV-6, and HHV-7 after lung transplantation.

MATERIALS AND METHODS

1. Patients

A total of 105 thoracic organ transplant recipients operated at Helsinki University Central Hospital were included in the studies (Table 5). Heart-lung transplant recipients (HLTRs) are included in the group of LTRs in the text, if not mentioned separately.

Antithymocyte globulin (ATG) (1.25 – 2.5 mg/kg/day) was given to all HTRs/HLTRs and 21 LTRs for 1-5 postoperative days (POD). The maintenance immunosuppressive regimen consisted of CyA (target trough level of 200-400 ng/ml), AZA 1-2 mg/kg/day or MMF 2-3 g/day, and methylprednisolone starting with 1 g perioperatively and tapered down to 0.1 mg/kg/day. One HTR received tacrolimus instead of CyA (Table 5). Acute rejection was treated with methylprednisolone 0,5-1 g daily for three days and with murine monoclonal antibody (OKT-3) or ATG if needed.

Antibiotic prophylaxis (usually third generation cephalosporins and/or vancomysin) was given for the immediate perioperative period to prevent bacterial infections. *Pneumocystis carinii* prophylaxis was initiated in 1992 and consisted of 1-2 tablets (160 mg of thrimethoprime and 800 mg of sulfamethoxazole) of Co-trimoxazole once daily three days a week or inhaled pentamidine every four weeks if the patient did not tolerate Co-trimoxazole. The prophylaxis was continued for six months in the 35 HTRs operated after 1992 and lifelong in all LTRs if tolerated. The antiviral prophylaxis is presented in the Section 3.3.

The chronological order of the studies I – IV is presented in Figure 6.

Table 5. The patient characteristics.

Total number of patients	105
Male/female	69/36
Age, mean (range)	46 (14 – 68)
Type of transplantation	
Lung	36 (34 %)
Heart-lung	20 (19 %)
Heart	49 (47 %)
Indication for lung or heart-lung transplantation	
α1-antitrypsin deficiency	11
Idiopathic pulmonary fibrosis	10
Congenital heart disease	10
COPD / Emphysema	8
Pulmonary hypertension	6
Lymphangioleiomyomatosis	3
Cystic fibrosis	2
Other[a]	6
Indication for heart transplantation	
Cardiomyopathy	27
Coronary artery disease	17
Myocarditis	3
Congenital heart disease	2
Maintenance immunosuppression	
CyA + AZA + Cs	76 (72 %)
CyA + MMF + Cs	28 (27 %)
Tac + AZA + Cs	1 (1 %)

[a] Bronchiectasis, Sdr. Kartagener, Cardiomyopathy, Bronchiolitis obliterans, Coronary artery disease, re-transplatation.
COPD, chronic obstructive pulmonary disease; CyA, Cyclosporine; AZA, Azathioprine; Cs, Corticosteroids; MMF, Mycophenolate mofetil; Tac, tacrolimus.

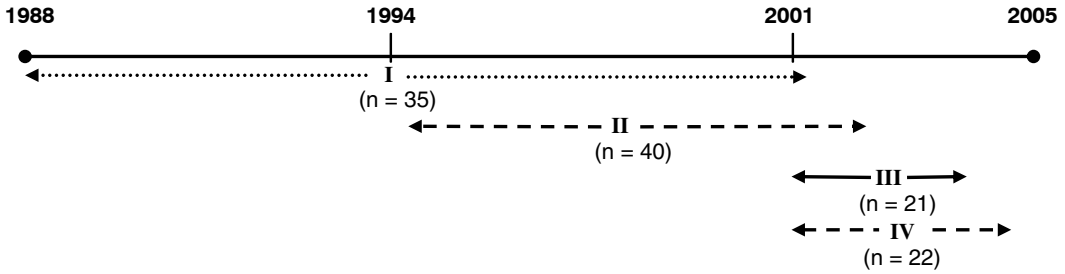

Figure 6. Timing of the transplantation and number of the patients in the Studies I – IV.

1.1. Bronchoscopies performed on heart and lung transplant recipients (I, II)

All the consecutive bronchoscopies performed at Helsinki University Central hospital on HTRs between May 1988 and December 2001 (Study I) and on LTRs between February 1994 and November 2002 (Study II) were analysed. Bronchoscopies with BAL were performed when a respiratory infection or rejection (LTRs) was suspected (clinically indicated bronchoscopies). In addition, LTRs were followed up by bronchoscopies performed weekly during the first postoperative month, then every 1-3 months during the first year posttransplant, and once a year thereafter (surveillance bronchoscopies). Transbronchial lung biopsy was performed on the LTRs one, three, six, nine, and 12 months after Tx and when acute rejection was suspected.

Thirty-five HTRs with a suspected lower respiratory tract infection underwent 47 bronchoscopies, while a total of 609 procedures were performed on 40 LTRs during the time periods studied. The number of bronchoscopies analysed in the diagnostic setting were 44 in HTRs and 472 in LTRs (190 clinically indicated and 282 surveillance bronchoscopies). The remaining bronchoscopies were follow-up procedures performed during the same episode of infection or rejection and did not yield any additional diagnostic information. However, all bronchoscopies were reviewed to detect potential complications of the procedure.

1.2. Lung and heart transplant recipients monitored by CMV antigenemia, DNAemia, and mRNAemia tests (III)

A total of 21 thoracic organ transplant recipients (seven LTRs and 14 HTRs) operated between December 2000 and April 2003 at Helsinki University Central Hospital were prospectively monitored with CMV antigenemia, DNAemia (PCR), and mRNAemia (NASBA) blood tests for a

12-month period. Blood samples were collected weekly during the hospital stay, once every two weeks until six months, and monthly during 6-12 months postoperatively. Additional blood samples were drawn if CMV infection was suspected or detected. Altogether 448 blood samples were received for CMV assays. Two (0.4 %), eight (1.8 %), and 25 (5.6 %) of the samples were not applicable or valid for the antigenemia test, PCR, and NASBA, respectively, and, due to long geographic distances, 28 blood samples could not be collected as required in the follow-up protocol.

1.3. Lung transplant recipients monitored for CMV, HHV-6, and HHV-7 (IV)

Twenty-two LTRs operated on at Helsinki University Central Hospital between December 2000 and October 2004 were prospectively monitored for the three β-herpesviruses (HHV-6, HHV-7, and CMV). Blood samples for detection of HHV-6, HHV-7, and CMV antigens were collected weekly during the hospital stay and at each outpatient visit thereafter. Additional blood samples were obtained if a viral infection was suspected or a CMV infection was detected. The three β-herpesviruses were also analysed from BALF. Altogether, 324 blood specimens (a median of 14 per patient; range 8-31) and 181 BALF samples (a median of eight per patient; range 6-11) were obtained.

2. Bronchoscopies

2.1. The bronchoscopic procedures

The fiberoptic or videobronchoscope was used for the bronchoscopies. Before BAL bronchial brushing with PSB was performed. BAL was performed by wedging the tip of the bronchoscope into the segmental bronchus of the area with the greatest radiologic or visual abnormality. A total of 160-200 ml of sterile physiologic saline was instilled in 20 ml aliquots. Gentle manual suction was applied to retrieve the saline. Transbronchial biopsies were performed only on the LTRs. TBBs were taken under fluoroscopic guidance from the areas of maximal radiologic infiltrate using alligator forceps. In case of no parenchymal infiltration the samples were taken from every lobe. Five to six TBB samples were collected per procedure.

2.2. Specimens received by the bronchoscopy

The BALF and PSB samples were cultured for bacteria and fungi. Cultures for mycobacteria, legionellae, and viruses as well as staining for fungi were made from BALF. Varicella zoster, Herpes simplex, *Legionella pneumophilia*, and common respiratory viruses were identified by antigen detection in BALF. Giemsa silver-methenamine stain from BALF and, since 1992, also antigen detection was used to detect *Pneumocystis carinii*. The demonstration of CMV is described in the Section 3.1. All BALF left from the microbiological studies was used for cytological investigations to determine the total and differential cell count, viral inclusion bodies, microbes, and atypical cells. The TBB specimens were examined histologically and for CMV-pp65 and *P. carinii* antigen expressions. In addition to these routine tests, HHV-6 and -7 were demonstrated from BALF in the Study IV, as described in the Section 4.

2.3. Significance of the findings received by the bronchoscopy

The bacteria in the BALF or PSB samples were considered significant if they were known respiratory pathogens and the patient suffered from a lower respiratory tract infection. The bacteria cultured from the surveillance bronchoscopic samples were considered significant if the same organism was later detected in BALF or PSB samples during symptomatic respiratory infection. In the LTRs colonizations with *Pseudomonas aeruginosa* or *Stenotrophomonas maltophilia* were considered clinically significant if an antibiotic therapy was initiated by the clinician.

In the LTRs the detection of Aspergillus species in the bronchoscopic samples was always regarded as significant. If characteristic membranes and ulcerations were detected together with Aspergillus airway colonization, the diagnosis was Aspergillus tracheobronchitis. Invasive Aspergillosis was defined as a histopathologic or cytopathologic finding showing hyphae associated with tissue damage (Ascioglu et al. 2002). The *Candida* species cultured from the bronchoscopic samples were not considered significant unless histopathologic evidence of invasive fungal pneumonia or positive blood culture was present.

Pneumocystis carinii and non-CMV viruses were always regarded as clinically significant pathogens when detected in the BALF or TBB specimens. The CMV pneumonia (disease) was defined as described in the Section 4.2.

The diagnosis and severity of acute rejection in the TBB specimens were defined according to the ISHLT criteria (Yousem et al. 1996). The anastomotic complication was defined as endobronchial stricture or granulation tissue resulting in symptoms or functional impairment of the recipient and necessitating an intervention.

3. CMV infection

3.1. Demonstration of CMV

CMV antigenemia test (I – IV)

The detection of CMV antigens in peripheral blood, BALF, and tissue specimens was based on the standard CMV pp65 antigen test (The et al. 1995). For detection of CMV antigenemia cytocentrifuge preparations were prepared onto microscope slides, after which a three-layer indirect immunoperoxidase technique and a monoclonal antibody against CMV pp65 antigen (Biotest, Frankfurt, Germany) were used (Fig. 7). CMV antigenemia in peripheral blood was measured by calculating CMV pp65 positive leukocytes per 50 000 PMNL on the slide.

CMV DNAemia (PCR) test (III)

The Cobas Amplicor CMV Monitor Test (Roche, Indianapolis, USA) was used for quantification of CMV DNA in the EDTA blood samples. The test was done according to the manufacturer's instructions and as described (Piiparinen et al. 2002 and 2004). The lower detection limit of the assay was 400 copies/ml and the linear range 400 - 100,000 copies/ml of plasma.

CMV mRNAemia (NASBA) test (III)

CMV mRNAemia was demonstrated by the NucliSens assay (bioMérieux, Boxtel, Netherlands) according to the manufacturer's instructions. The assay uses a qualitative nucleic acid sequence-based amplification (NASBA) to detect pp67 mRNA, and the results are reported as positive or negative.

Demonstration of CMV in BALF and tissue specimens

CMV was demonstrated in the BALF and tissue specimens (e.g. TBB) by immunohistochemistry using the detection of CMV pp65antigen-positive cells or by detecting characteristic intracellular inclusion bodies (Ljungman et al. 2002a). In addition, rapid shell vial cultures were performed from the BALF samples to detect CMV.

3.2. Diagnosis of CMV infection

CMV infection was defined as a positive CMV antigen test in blood or BALF. In the Study III CMV infection was also demonstrated by the CMV DNAemia and mRNAemia tests. However, only the CMV antigenemia test was used to guide the antiviral therapy.

The diagnosis of the *CMV disease* (e.g. pneumonia) was confirmed when symptoms and/or findings referring to a viral infection were present in combination with the detection of characteristic intracellular inclusion bodies or CMV pp65-antigen in tissue specimens or BALF. However, sole CMV pp65-antigen positive cells in BALF were not accepted for the diagnosis of the CMV disease in the Study II, though this finding was considered significant in terms of demonstrating CMV infection. In that study, one of the aims was to test the usefulness of the CMV antigen test and culture to detect otherwise confirmed CMV pneumonia.

3.3. Prevention and treatment of CMV infection

Antiviral prophylaxis against CMV for a minimum of three postoperative months was given to the LTRs since January 1995 if the recipient or donor was seropositive (R+ or D+). Valganciclovir was used as CMV prophylaxis to one HTR (R-/D+) in the Study III, while all the other HTRs and LTRs

at low risk of CMV infection (R-/D-) received oral acyclovir (600 mg/day) for three postoperative months. The routine prophylactic strategy changed over the study years, as orally administered ganciclovir and valganciclovir became available. The length and dosing of the prophylaxis also varied due to the side-effects of the drugs, the renal function of the recipient, and the antirejection therapies needed. Details of the pretransplant CMV-serostatus and prophylaxis against CMV in the prospective studies (III and IV) are presented in Table 6.

Table 6. The pretransplant CMV-serostatus and prophylaxis against CMV in the Studies III and IV.

Factor	Study III	Study IV
Pretransplant CMV-serostatus		
R+/D+	14 (67 %)	14 (63 %)
R+/D-	6 (28 %)	4 (18 %)
R-/D+	1 (5 %)	1 (4 %)
R-/D-	0 (0 %)	3 (14 %)
CMV prophylaxis		
iv.GAN/po.GAN[a] (7-90 POD)	3	3
po.GAN[b] (7-90 POD)	2	2
po. valGAN[c] (7-90 POD)	2	2
po. valGAN[c] (7-120 POD)		4
po. valGAN[c] (7-180 POD)	1	5
po. valGAN[c] (7-210 POD)		2
po. valGAN[c] (7-270 POD)		1
No prophylaxis[d]	13[e]	3[f]

CMV, Cytomegalovirus; R, Recipient; D, Donor
GAN, ganciclovir; POD, postoperative days; valGAN, valganciclovir
[a] Iv. GAN 10 mg/kg/day 7-21 POD, 5 mg/kg/day 22-28 POD and po. GAN 3g/day 29-90 POD
[b] 3g/day (The doses of GAN were adjusted for renal function)
[c] 450-900 mg/day (The dose was adjusted for renal function and body weight)
[d] Acyclovir (600 mg/day) until POD 90 was given to prevent other herpesvirus diseases
[e] Heart transplant recipients
[f] Lung transplant recipients at low risk of CMV infection (R-/D-)

In addition to the universal prophylaxis, a pre-emptive antiviral therapy against CMV infection was increasingly used from the late 1990s based on the results of the CMV antigenemia test. Before the year 2001 the decision of initiating pre-emptive therapy was made by the attending physician. In the

prospective studies (III and IV) the pre-emptive treatment strategy was as follows: Antiviral therapy was initiated with pp65-positive leukocytes ≥2 and ≥10 per 50 000 PMNL in the LTRs and HTRs, respectively. HTRs with an antigenemia level from 5 to 9 pp65-positive leukocytes received antiviral therapy immediately or the assay was controlled in one week and the therapy was initiated if increasing antigenemia was detected. All patients with symptomatic CMV infection or CMV disease received antiviral therapy regardless of the antigenemia level (rescue therapy). Intravenous ganciclovir (5 mg/kg b.i.d.) or peroral valganciclovir (900 mg b.i.d.) was used as antiviral therapy for CMV infection. The treatment was continued for a minimum of 14 days and until the CMV antigenemia test was negative.

4. Demonstration of HHV-6 and HHV-7 (IV)

HHV-6 and HHV-7 were demonstrated by detecting the virus-specific antigens in the PBMCs and BALF cells (Fig. 7). The PBMCs were isolated by Ficoll-Isopaque density gradient centrifugation and cytocentrifuged onto microscope slides. The presence of HHV-6 antigens in the cytopreparations of the PBMCs and BALF cells was demonstrated by indirect immunoperoxidase staining and by monoclonal antibodies (MAB8533 and MAB8535; Chemicon Inc., Temecula, CA, USA) against an early HHV-6 specific antigen and an HHV-6 variant B virion protein, as described (Lautenschlager et al. 2000). Normal mouse-IgG was used as negative control for non-specific binding. A peroxidase-conjugated rabbit anti-mouse (Dako, Copenhagen, Denmark) antibody and a peroxidase-conjugated goat anti-rabbit antibody (Zymed, San Francisco, CA, USA) were used as second and third antibodies. The reaction was revealed by 3-amino-9-ethyl carbazole solution containing hydrogen peroxide, and Mayer's haemalum was used for counterstaining. Concomitantly, HHV-7-specific antigens were demonstrated in the cytocentrifuge preparations by using immunoperoxidase staining with monoclonal antibodies (Biodesign International, Saco, ME,

USA), as described (Lautenschlager et al. 2002). No cross-reactivity between the antibodies or non-specific staining was recorded.

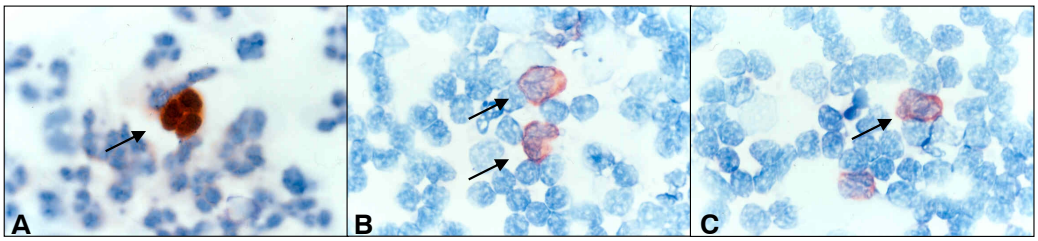

Figure 7. CMV pp65-antigen-positive granulocyte (A). Human herpesvirus-6 (B) and human herpesvirus-7 (C) antigen-positive lymphocytes. Positive cells are pointed with arrows.
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5. Statistical Analyses

The diagnostic yield of bronchoscopy in different time intervals and patient groups was compared using the chi-square test. The time-related appearance of each β-herpesvirus and the time to the first CMV infection in recipients with and without prophylaxis were compared using the Kaplan-Meier method. Differences between the groups were analysed by the log-rank test. The number of CMV pp65-antigen-positive leukocytes and the CMV DNAemia level were compared using Spearman´s rank correlation test. The CMV infections positive by different assays in the Study III and the patient groups with different CMV prophylaxis in the Study IV were compared by Fisher's exact test. Receiver-operating characteristic (ROC) curves were performed to determine the optimal threshold CMV DNAemia levels for initiation of the pre-emptive antiviral therapy when different CMV antigenemia cut-off levels were used as a reference. A statistical significance was accepted for $p < 0.05$.

RESULTS

1. Utility of bronchoscopies (I, II)

The overall diagnostic yield of the clinically indicated bronchoscopies was 18/44 (41 %) and 115/190 (61 %) in the HTRs and LTRs, respectively. When only the microbiological diagnosis of infection was considered, the diagnostic yield for the HTRs remained 41 %, while it was 51 % in the LTRs. The procedure caused a change in the medical therapy in 105/190 (55 %) and 14/44 (32 %) of the clinically indicated bronchoscopies performed on the LTRs and HTRs, respectively. Surveillance bronchoscopies performed on the LTRs established a diagnosis in 43/282 (15 %) of the procedures, and the findings led to a change in the previous treatment of the patient in 16/282 (6 %) of the cases. The diagnostic yield of bronchoscopy was highest from one to six months posttransplant when compared to all the other time intervals in both the HTRs and LTRs ($p < 0.05$). The overall diagnostic yield was 26/70 (37 %) and 8/92 (9 %) for TBBs performed on the LTRs in the clinically indicated and surveillance bronchoscopies, respectively ($p < 0.001$). None of the surveillance TBBs, but 19/70 (27 %) of the clinically indicated TBBs directly changed the drug therapy of the recipient.

2. Diagnoses established by the bronchoscopy (I, II)

The diagnoses established by the bronchoscopy and significant microbes detected in the bronchoscopic specimens are presented in Table 7.

Table 7. Diagnoses established by the bronchoscopy in HTRs and LTRs. From two to four diagnoses were established by one bronchoscopy in 4/44 (9 %) and 40/190 (21 %) of the bronchoscopies performed on HTRs and LTRs, respectively.

	HTRs cFBs (n = 44)	LTRs cFBs (n = 190)	LTRs sFBs (n = 282)
Infection	**18 (40.9 %)**	**96 (50.5 %)**	**34 (12.1 %)**
P. carinii	9 (20.5 %)	18 (9.5 %)	5 (1.8 %)
CMV	9 (20.5 %)	27 (14.2 %)	7 (2.5 %)
Bacteria	1 (2.3 %)	57 (30.0 %)	9 (3.2 %)
P aeruginosa		11	5
P aeruginosa + Str.pneumoniae		1	
Stenotroph. maltophilia		7	3
Staph. aureus		10	
Staph. aureus + Str. pneumoniae		1	
Str. pneumoniae		6	
H influenzae		3	1
M catarrhalis		4	
H influenzae + M catarrhalis	1		
Nocardia		3	
Staph. epidermidis		3	
Klebsiella oxytoca		2	
L pneumophila		2	
Other [a]		4	
Aspergillus	2 (4.5 %)	7 (3.7 %)	10 (3.5 %)
Viruses other than CMV	1 (2.3 %)	11 (5.8 %)	6 (2.1 %)
HSV		1	2
VZV			1
RSV	1	4	
RSV + VZV		1	
Influenzavirus		2	2
Parainfluenzavirus		2	
Parainfluenzavirus + HSV		1	
Adenovirus			1
Mycobacteria		2 (1.1 %)	
Acute rejection		**21 (11.1 %)**	**8 (2.8 %)**
Grade 1		10	8
Grade ≥ 2		11	
Airway complication		**12 (6.3 %)**	**1 (0.4 %)**
Other [b]	**1 (2.3 %)**	**4 (2.1)**	
No diagnosis	**26 (59.1 %)**	**75 (39.5 %)**	**239 (84.8 %)**

HTR, heart transplant recipient; LTR, lung transplant recipient; cFB, clinically indicated flexible bronchoscopy; sFB, surveillance flexible bronchoscopy

[a] *E faecalis, E coli, S marcescens*, Mycoplasma

[b] Endobronchial adenocarcinoma (HTR), Eosinophilic pneumonia (2), COP, bronchiolits obliterans.

Pneumocystis carinii was detected in 9/44 (20 %) and 23/472 (5 %) of the bronchoscopic specimens from the HTRs and LTRs, respectively. In the LTRs 15/23 (65 %) of the *P. carinii* infections were breakthrough infections detected during adequate chemoprophylaxis (eight during co-trimoxazole and seven during inhaled pentamidine). The characteristics of the episodes with *P. carinii* detected in the bronchoscopic samples from the LTRs are presented in Table 8. In the 21 HTRs who had been given prophylaxis two episodes of *P. carinii* infections occurred two and four months after finishing the chemoprophylaxis.

Table 8. Pneumocystis carinii detected in bronchoscopic specimens in lung transplant recipients.

	P. carinii prophylaxis			
	TMP-SMZ	IP	None	**Total (% of P.carinii infections)**
No.	11	8	4	**23 (100 %)**
Type of FB				
Clinically indicated FB	9	5	4	**18 (78 %)**
Surveillance FB	2	3	0	**5 (22 %)**
Infiltrate on HRCT or chest radiograph				
Infiltrate	7	4	2	**13 (57 %)**
No Infiltrate	4	4	2	**10 (43 %)**
Time after transplantation				
< 30 days	3	0	2	**5 (22 %)**
31-360 days	5	3	0	**8 (35 %)**
361-720 days	2	3	1	**6 (26 %)**
> 720 days	1	2	1	**4 (17 %)**
P. carinii detected in				
BALF + / TBB not done	7	4	3	**14 (61 %)**
BALF + / TBB +	1	1	1	**3 (13 %)**
BALF + / TBB -	1	3	0	**4 (17 %)**
BALF - / TBB +	2	0	0	**2 (9 %)**

TMP-SMZ, Co-trimoxazole; IP, inhaled pentamidine, FB flexible bronchoscopy, HRCT, high-resolution computed tomography, BALF, bronchoalveolar lavage fluid, TBB, transbronchial lung biopsy.
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Cytomegalovirus was detected by the pp65-antigen test or viral inclusions in the specimens from 34/472 (7 %) of the bronchoscopies performed to LTRs. The positive predictive values for CMV pneumonia by the CMV antigen test and the CMV culture in the BALF from the symptomatic recipients (clinically indicated bronchoscopy) were 24.0 % and 9.7 %, respectively. In addition, CMV was detected in the BALF from the surveillance bronchoscopies by the antigen test in 7/282 (3 %) and by the culture in 63/282 (22 %) of the cases. Nine cases of CMV pneumonia were detected in the HTRs carrying a mortality rate of 44 % (4/9). Bacteria were the most frequently detected significant microbes in the LTRs, while the microbiological diagnosis of bacterial pneumonia was received in only one HTR. However, 73 % of the HTRs were treated with empiric antibiotics before the bronchoscopy. *Aspergillus* species detected in the bronchoscopic samples were related to histologically proven invasive A*spergillus* infection in four recipients (two LTRs and two HTRs). All the other cases revealed *Aspergillus* airway colonization in the LTRs. None of the *Candida* species cultured from the bronchoscopic samples were related to invasive Candida infection. All grade 2-3 and 6/10 of the grade 1 symptomatic rejections detected in the clinically indicated TBBs were treated with antirejection therapy, while no therapy was initiated for the eight asymptomatic rejections (grade 1) diagnosed by surveillance TBBs.

3. Safety of the bronchoscopies (I, II)

There were no fatalities associated with the bronchoscopies. The procedure provoked respiratory insufficiency in three and myocardial ischaemia in one of the HTRs. Five LTRs suffered from respiratory insufficiency demanding ventilatory assistance, and one LTR developed severe hypoxemia disturbing the procedure. Four bronchoscopies were prematurely interrupted due to bleeding in the LTRs. Two spontaneously resolving pneumothoraces and one local pulmonary haemorrhage were detected after TBB. Thus, 17 clinically significant complications were detected

giving a complication rate of 2.1 % and 9.1 % for the bronchoscopies performed on the LTRs and HTRs, respectively. In addition, minor bleeding was occasionally observed during the procedure, but all bleeding episodes resolved without interventions and none led to hypotension or blood transfusion.

4. CMV antigenemia, DNAemia, and mRNAemia in lung and heart transplant recipients (III)

CMV antigenemia, DNAemia, and mRNAemia were detected in 21/21 (100 %), 20/21 (95 %), and 13/21 (62 %) of the thoracic organ transplant recipients, respectively. A total of 46 CMV infections, defined as a positive result in any of the tests, occurred in the study population. CMV infection was detected by the antigenemia, DNAemia, and mRNAemia tests in 45/46 (98 %), 33/46 (72 %), and 20/46 (43 %) of the cases, respectively. All the PCR positive infections were also detected by the antigenemia test, while CMV DNAemia was not detected in 12 (27 %) of the CMV antigenemias. However, all the antigenemias without concomitant DNAemia manifested as a low level of pp65-positive leukocytes (≤ 5/50 000) and only two of these episodes required antiviral therapy. A significant correlation between the number of CMV pp65-positive leukocytes and the CMV DNAemia level was found ($r = 0.69$, $p < 0.0001$).

5. Usefulness of CMV DNAemia and mRNAemia tests in guiding antiviral therapy (III)

CMV DNAemia was detected in 26/28 (93 %) and mRNAemia in 17/28 (61 %) of the CMV antigenemias requiring antiviral therapy ($p = 0.01$). The two PCR negative CMV antigenemias treated with antiviral agents manifested as a low peak antigenemia level of 2 and 5 pp65-positive leukocytes, respectively. Furthermore, CMV DNAemia was detected before or at the initiation of the therapy in all of the 26 PCR positive CMV antigenemias. Sensitivity, specificity, and positive

predictive values (PPV) of the different DNAemia levels and NASBA results using the antigenemia test as the reference standard are presented in Table 9.

Table 9. Sensitivity, specificity, and positive predictive values (PPV) of the different DNAemia levels (PCR) and mRNAemia (NASBA) test results using the antigenemia test as the reference. Blood samples collected during antiviral therapy were excluded.

	Threshold levels of CMV antigenemia		
	≥ 2 pp65-positive leukocytes	≥ 5 pp65-positive leukocytes	≥ 10 pp65-positive leukocytes
Threshold levels of DNAemia (PCR)			
> 400 copies/ml			
Sensitivity (%)	75.9	91.3	100
Specificity (%)	92.7	87.2	85.7
PPV (%)	66.1	33.9	24.2
≥ 1000 copies/ml			
Sensitivity (%)	61.1	87.0	100
Specificity (%)	95.5	91.9	90.5
PPV (%)	71.7	43.5	32.6
≥ 5000 copies/ml			
Sensitivity (%)	24.1	47.8	66.7
Specificity (%)	100	99.4	99.1
PPV (%)	100	84.6	76.9
Optimal DNAemia level[a]	400 copies/ml	850 copies/ml	1250 copies/ml
Sensitivity (%)	75.9	91.3	100
Specificity (%)	92.7	91.3	91.5
PPV (%)	66.1	42.9	34.9
mRNAemia (NASBA)			
Sensitivity (%)	25.9	43.5	56.3
Specificity (%)	99.6	98.4	98.1
PPV (%)	93.3	66.7	60.0

[a] Optimal DNAemia levels from the receiver operating characteristic (ROC) curves were chosen as the point nearest to the left top corner in order to achieve the maximal sum of sensitivity and specificity.
CMV, Cytomegalovirus.
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6. HHV-6 and HHV-7 activation in lung transplant recipients (IV)

During the first posttransplant year HHV-6, HHV-7, and CMV antigenemia were detected in 20/22 (91 %), 11/22 (50 %), and 12/22 (55 %) of the LTRs, respectively. After Tx, HHV-6 antigenemia first appeared in a median of 16 (range 9 - 232) days, HHV-7 antigenemia in a median of 31 (range 1 - 198) days, and CMV antigenemia in a median of 165 (range 94 - 323) days. The cumulative incidences of the beta-herpesviruses are shown in Figure 8.

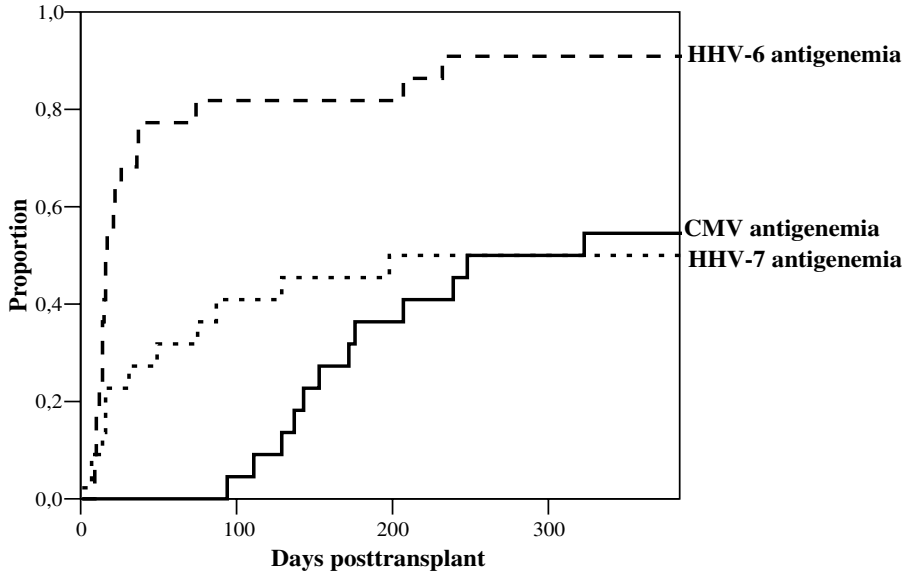

Figure 8. Log-rank curves showing the timing of the first detection of HHV-6, HHV-7, and CMV antigenemia in 22 lung transplant recipients. HHV-6 vs. CMV ($p < 0.001$) and HHV-6 vs. HHV-7 ($p < 0.01$). Ganciclovir or valganciclovir prophylaxis was given to 19/22 (86 %) of the patients (IV).
HHV 6, Human herpesvirus 6; HHV 7, Human herpesvirus 7; CMV, Cytomegalovirus.
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HHV-6, HHV-7, and CMV antigens were detected in 170/324 (52.5 %), 57/324 (17.6 %), and 42/324 (13.0 %) of the blood specimens and in 8/181 (4.4 %), 1/181 (0.6 %), and 3/181 (1.7 %) of the BALF samples, respectively. Seven out of the nine BALF samples showing HHV-6 or HHV-7 antigens were microscopically blood-contaminated, and in eight cases HHV-6 or HHV-7 was

detected concomitantly in the blood and BALF. HHV-6 was detected concomitantly in 12/18 (67 %) and HHV-7 in 6/18 (33 %) of the episodes of CMV antigenemia.

One case of mild pneumonitis was possibly caused by HHV-6 and another recipient had encephalitis associated with the appearance of HHV-6 antigenemia. Neither of these recipients received CMV prophylaxis (R-/D-). All the other episodes of the HHV-6 or HHV-7 antigenemias were either asymptomatic or the possible manifestations could not be distinguished from those of the concomitant infections (e.g. CMV), drug side-effects or complications of the immediate postoperative period.

7. Efficacy of the antiviral prophylaxis against CMV, HHV-6, and HHV-7 antigenemia (III, IV)

In the Study III, the first detection of CMV antigenemia was delayed in the patients receiving CMV prophylaxis (median of 137 days) when compared to the recipients without the prophylaxis (median of 23 days).

The incidences of HHV-6, HHV-7, and CMV antigenemia in 19 recipients receiving ganciclovir or valganciclovir as antiviral prophylaxis against CMV in the Study IV are shown in Table 10. Induction therapy with ATG was given to all five recipients with ganciclovir prophylaxis, but only to one patient with valganciclovir prophylaxis. All three CMV-seronegative recipients having a graft from a seronegative donor (R-/D-) developed HHV-6 antigenemia and two HHV-7 antigenemia during the acyclovir prophylaxis, but none suffered from a primary CMV infection. The concomitant HHV-6 and HHV-7 antigenemia responded to the antiviral therapy directed against CMV in terms of disappearance of the virus from blood in 9/12 (75 %) and 5/6 (83 %) of

the cases, respectively. However, the efficacy of the therapy against HHV-6 and HHV-7 was often delayed and less clear when compared to that of CMV antigenemia.

Table 10. The incidences of HHV-6, HHV-7, and CMV antigenemia in lung transplant recipients with antiviral prophylaxis against CMV.

	Total (n = 19)	GAN (n = 5)	ValGAN (n = 14)	P-value [a]
Breakthrough antigenemia during prophylaxis				
HHV-6	**15/19 (79 %)**	4/5 (80 %) [b]	11/14 (79 %)	p > 0.05
HHV-7	**7/19 (37 %)**	4/5 (80 %) [b]	3/14 (21 %)	p = 0.04
CMV	**1/19 (7 %)**	0/5 (0 %)	1/14 (7 %)	p > 0.05
Total incidence of antigenemia				
HHV-6	**17/19 (89 %)**	5/5 (100 %)	12/14 (86 %)	p > 0.05
HHV-7	**9/19 (47 %)**	5/5 (100 %)	4/14 (29 %)	p = 0.01
CMV	**12/19 (63 %)**	5/5 (100 %)	7/14 (50 %)	p > 0.05

HHV 6, Human herpesvirus 6; HHV 7, Human herpesvirus 7; CMV, Cytomegalovirus; GAN, ganciclovir; valGAN, valganciclovir

[a] GAN vs. valGAN prophylaxis

[b] Three HHV-6 and HHV-7antigenemias appeared during p.o. and one during i.v. ganciclovir

DISCUSSION

1. Usefulness of bronchoscopy

The diagnostic yield of BAL has varied largely, from 27 to 69 %, in immunocompromised hosts including SOT recipients (Cazzadori et al. 1995, Torres et al. 2000, Rano et al. 2001, Reichenberger et al. 2001, Jain et al. 2004). In the only report evaluating the use of bronchoscopy solely in HTRs (Schulman et al. 1998) the diagnostic yield was somewhat higher (62 %) than that found in the present study (41 %). Two factors, probably increasing the diagnostic yield in the Schulman study, were the frequently performed TBBs and the absence of Pneumocystis prophylaxis (Schulman et al. 1988). Clinically indicated bronchoscopies performed on the LTRs revealed a specific diagnosis in 61 % of the cases in the present study, the majority of the diagnoses being respiratory infections. The higher diagnostic yield of bronchoscopy in the LTRs compared to that in the HTRs can be explained by the high frequency of infections as well as by other postoperative complications (e.g. rejection) in the lung allograft. The diagnostic yield of clinically indicated bronchoscopy in the present study is in line with that of previous reports on LTRs (Chan et al. 1996, Baz et al. 1996). The utility of bronchoscopy was highest from one to six months after Tx in both the HTRs and LTRs. This is suggested to be mainly due to the high occurrence of opportunistic respiratory infections as well as to acute rejections (LTRs) during this time interval.

There are two general issues which have to be addressed to when the usefulness of bronchoscopy in the diagnosis of a suspected respiratory infection is evaluated. Firstly, the sensitivity or specificity of bronchoscopy is difficult to determine due to the lack of “the gold standard” for the diagnosis of pneumonia. Nevertheless, although the widely used term “diagnostic yield” (the proportion of the procedures by which a specific diagnosis is achieved) is not equal to sensitivity, it provides valuable

information of the usefulness of bronchoscopy in different clinical settings. Secondly, as approximately half of the SOT recipients with a suspected respiratory infection may not receive the diagnosis through bronchoscopy, its role as recommended diagnostic procedure could be questioned. However, the usefulness of bronchoscopy has to be weighted against the low yield of non-invasive procedures (e.g. blood cultures or sputum analysis) and the risks of performing open lung biopsy in immunocompromised patients (Nusair and Kramer 1999, Chang et al. 2004, Shorr et al. 2004). Thus, bronchoscopy is a valuable procedure for the diagnosis of respiratory infections in transplant recipients compared to other available diagnostic methods.

More than half of the Tx centres perform regularly scheduled surveillance bronchoscopies on asymptomatic LTRs in order to detect clinically silent rejection or infection (Kukafka et al. 1997). Only 15 % of the surveillance bronchoscopies established a specific diagnosis in the present material, which is a slightly lower yield than that received in most previous reports (Baz et al. 1996, Hopkins et al. 2002, Chakinala et al. 2004). It can be explained by two factors. Firstly, the occurrence of acute rejection was low in the present material with none of the surveillance TBBs establishing grade ≥ 2 acute rejection. This reflects a relatively high net state immunosuppression of the LTRs in the Study II (all patients received ATG). Secondly, the routine surveillance of the LTRs by chest CT scan led to rather strict criteria for surveillance bronchoscopies: the procedures were classified as clinically indicated when changes were detected in the CT scan even in asymptomatic recipients with clear plain chest radiography. Nevertheless, since the surveillance bronchoscopies seldom changed the medical therapy of the patient, the present author agrees with some of the previous studies questioning the role of surveillance bronchoscopies in the management of LTRs (Tamm et al. 1997, Valentine et al. 2002).

All the major complications of the bronchoscopy in the HTRs were cardiovascular and occurred in patients with a compromised cardiovascular condition. According to international recommendations, bronchoscopy should be avoided in patients with poorly controlled congestive heart failure or a history of recent myocardial infarction (Honeybourne et al. 2001). This recommendation is difficult to follow in HTRs. In LTRs, the complication rate of bronchoscopy in the present study (2.1 %) was lower than that in most previous studies, but similar to the rate (2.0 %) reported by Dransfield and co-workers (Baz et al.1996, Boehler et al. 1996, Hopkins et al. 2002, Dransfield et al. 2004). In the present study, the bleeding volume was not routinely calculated after a minor bleeding. In addition, TBBs were taken in only 32 % of the bronchoscopies performed on the LTRs. These factors probably lower the present complication rate compared to that of previous reports.

2. Significance of microbes retrieved by bronchoscopy

Pneumocystis carinii was a frequently detected pathogen in the BALF in the present study. This finding is expectable in patients who underwent HTx before the institution of chemoprophylaxis. However, the relatively high frequency of *P. carinii* in the LTRs who were receiving the prophylaxis is surprising, since Co-trimoxazole and inhaled pentamidine are highly effective in preventing PCP (Schneider 1992, Nathan et al. 1994, Gordon et al. 1999). *P. carinii* was detected by surveillance bronchoscopy in five cases, while all the other LTRs with *P.carinii* demonstrated in the BALF or TBBs had respiratory symptoms and/or radiographic infiltrates. Thus, although some of the cases may have represented the result of airborne transmission of the organism or colonization of the recipient, it is likely that most of the recipients had a clinical infection. Significant mortality rates of about 30 - 40 % in PCP have been reported in patients without prophylaxis (Gordon et al. 1999, Roblot et al. 2002). In contrast, all patients in the present study

responded to the therapy, suggesting that previous chemoprophylaxis may modify the clinical picture of PCP. The optimal length of the prophylaxis against *P. carinii* in SOT recipients remains a matter of debate (Fishman and Rubin 1998, Gordon et al. 1999, Villician and Paya 1999). In the present patients, P. carinii was detected in the LTRs from the immediate postoperative period to more than two years post-transplantation, and two HTRs suffered from PCP soon after discontinuation of the Co-trimoxazole prophylaxis. Thus it is reasonable to give lifelong *P. carinii* prophylaxis for LTRs, as stated by Gordon and co-workers (Gordon et al. 1999). Nevertheless, *P.carinii* infections still occur in LTRs despite the chemoprophylaxis, and bronchoscopy is warranted in the diagnosis and surveillance of this organism. The present results also support the recommendations to expand the length of the prophylaxis beyond six months in HTRs, especially when the level of immunosuppression is intensified (Villician and Paya 1999, Fishman 2001).

Detecting CMV by the antigen test or culture in the BALF had a poor positive predictive value in the LTRs, when rather strict criteria were used for the diagnosis of CMV pneumonia. Similarly, a limited impact of CMV culture or antigen detection from the BALF on medical treatment has been detected in bone marrow transplant recipients (Ruutu et al. 1990, Feinstein et al. 2001). However, in two recent studies the quantitative measurements of CMV DNA from BALF are suggested to be more predictive of CMV pneumonia than the screening of CMV DNAemia from blood on the LTRs (Westall et al. 2004, Chemaly et al. 2005). This method could serve as a good alternative in the demonstration of CMV in BALF.

In the present study, bacteria were the most common causative agents for respiratory infection in the LTRs. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common bacteria, which is in line with the international reports (Trulock 1999). In contrast, the diagnostic accuracy of the present bronchoscopic samples for bacterial pneumonia was low in the HTRs. The most likely

reason for this is the empiric antibiotic therapy which was widely used at the time of the bronchoscopy. Furthermore, the use of quantitative cultures could have increased the diagnostic yield of the bronchoscopy by detecting the high growth of bacteria which was interpreted as colonization the present study.

Aspergillus airway colonization is regarded as a risk factor for anastomotic complications as well as for an invasive disease in LTRs (Nathan et al. 2000, Cahill et al. 1997). The present results could not show any clear association between aspergillus colonization and anastomotic complications in the LTRs. This can be partly explained by the low number of patients in the present study and by the use of the pre-emptive antifungal therapy in some of the patients by the discretion of the physician.

3. CMV DNAemia and mRNAemia and their relationship to antigenemia

The antigenemia and DNAemia (PCR) tests proved to be more sensitive in detecting CMV infection than the mRNAemia (NASBA) assay in the present study. The inability of NASBA to guide the antiviral therapy was demonstrated by two factors. Firstly, 39 % of the CMV antigenemia episodes requiring antiviral therapy were not detected by NASBA. Secondly, the sensitivity of the assay was low (25.9 - 56.3 %) in detecting the CMV antigenemia level of 2-10 pp65-positive leukocytes. In contrast to the present results, Gerna and coworkers concluded in their study that the antigenemia test could be replaced by NASBA in the guidance of the pre-emptive therapy in thoracic organ transplant recipients. However, a higher threshold level for the initiation of the therapy (100 pp65-antigen positive leukocytes /200 000 PMNLs) was used compared to the present study (Gerna et al. 2003).

The CMV DNAemia levels measured by The Cobas Amplicor PCR assay showed a good overall correlation with the pp65-antigenemia test results in the present material, which is in line with previous reports on SOT recipients (Caliendo et al. 2002, Pang et al. 2003). In order to evaluate the clinical usefulness of this correlation, the ROC curve analysis was performed to estimate the cut-off levels for CMV DNAemia corresponding to the antigenemia levels used for the initiation of the pre-emptive therapy. The optimal cut-off levels of CMV DNAemia to achieve maximal combined sensitivity and specificity were 850 and 1250 copies/ml for the antigenemia levels of 5 and 10 pp65-positive leukocytes, respectively. The sensitivities and specificities with these DNAemia tresholds were over 90 %, but the PPVs were low. Thus, using the above-mentioned cut-off levels for the initiation of the pre-emptive therapy all of the CMV antigenemias over 10 pp65-positive leukocytes and a great majority of antigenemias over 5 pp65-positive leukocytes would be treated, but if the antiviral therapy is initiated with DNAemia levels from ca. 1000 to 5000 copies/ml, some of the CMV infections might actually represent an antigenemia under 5-10 pp65-positive leukocytes. The sensitivity of any positive PCR result for an antigenemia level of 2 or more pp65-positive leukocytes was 75.9 %. If this lowest antigenemia level is used for the initiation of the pre-emptive therapy, the best alternative to the pp65-antigenemia test might be one of the real-time PCR assays, which, in the previous studies, has been even more sensitive than the Cobas Amplicor PCR test and the antigenemia assay (Pang et al. 2003, Piiparinen et al. 2004, Herrmann et al. 2004).

Although the antigenemia test was slightly more sensitive than the PCR assay in the present study, the differences between these two assays were not considered to be of major clinical significance. All the PCR-negative CMV infections manifested as low-level antigenemia; only two of the CMV infections requiring antiviral therapy were PCR-negative, and DNAemia was present until the initiation of the therapy in all the PCR-positive cases. Furthermore, the antigenemia test was arbitrarily selected for the reference assay, because it was traditionally used to demonstrate CMV

infection and guide pre-emptive therapy. It has to be noted, however, that the absolute superiority of the antigenemia, DNAemia or even mRNAemia assay over each other in detecting clinically significant CMV infection falls beyond the scope of the present study.

There are some limitations in the Study III. Firstly, there were differences in the prevention and treatment of the CMV infections between the HTRs and LTRs (prophylaxis and thresholds for pre-emptive therapy). This may weaken the clinical relevance of the conclusions made, when considered for each transplant group separately. However, the two other CMV assays could be compared to the antigenemia test in general by calculating the sensitivities and specificities for the different cut-off levels of antigenemia. Secondly, some of the blood samples could not be retrieved to the laboratory in time to be valid for the CMV assays. Long geographical distances are considered one of the major problems in collecting samples for the CMV tests and applying the pre-emptive treatment strategy (Singh 2001, Zamora 2004a). Thus, CMV tests that could be reliably performed also in distant centres (e.g. based on commercially available PCR) are needed.

4. HHV-6 and HHV-7 antigenemia in lung transplant recipients

In the present study a high incidence of HHV-6 antigenemia (91 %) and a lower incidence of HHV-7 antigenemia (50 %) were found after LTx during the first posttransplant year. In previous studies on SOT recipients the HHV-6 occurred in 0 - 66 % and HHV-7 in 42 - 89 % of the patients (Griffiths et al. 1999, Brennan et al. 2000, Lautenschlager et al. 2000, Des Jardin et al. 2001, Mendez et al. 2001, Michaelides et al. 2002, Jacobs et al. 2003, Razonable et al. 2005). Similarly to the present study, activation of both viruses has usually been detected during the first postoperative month after Tx (Brennan et al. 2000, Humar et al. 2002, Jacobs et al. 2003). There are two main reasons for the wide variation in the incidences reported. Firstly, the organ transplanted influences

the risk of viral infection through the differences in the immunosuppressive therapy. Secondly, the diagnostic method differs between the studies. However, the HHV-6 activation was detected in the present study more frequently than in previous studies on SOT recipients. It was shown in a recent study that HHV-6 antigenemia and DNAemia correlate well in liver transplant recipients (Härmä et al. 2005). Thus it is suggested that the relatively high incidence of HHV-6 in the present study is not entirely related to the method used. The relatively high occurrence of CMV antigenemia and the possible higher net-state immunosuppression, at least when compared to other SOT recipients than LTRs, may have influenced the high incidence of HHV-6 in the present patients. In a few reports of HHV-7 in SOT recipients, the incidence of the virus has been comparable to that of the present study (Griffiths et al. 1999, Mendez et al. 2001, Razonable et al. 2005). HHV-6 and, to a lesser extent, HHV-7 were frequently detected concomitantly or preceding the CMV antigenemia. This is in concordance with previous studies and suggests interactions between the viruses (Des Jardin et al. 2001, Lautenschlager et al. 2002, Razonable et al. 2003).

In the present study it was not possible to confirm any symptoms or findings to be solely due to HHV-6 or HHV-7. The manifestations of the viral activations were either asymptomatic or difficult to differentiate from those of other posttransplant complications (e.g. CMV or other infections). However, one case of mild pneumonitis was possibly caused by HHV-6, and another recipient had encephalitis associated with HHV-6 antigenemia. Neither of these recipients received CMV prophylaxis. Although most of the HHV-6 and HHV-7 activations have been asymptomatic also in other studies, the possibility that antiviral prophylaxis modifies the clinical picture of HHV-6 or HHV-7 infection cannot be ruled out (Emery 2001, Humar et al. 2002, Jacobs et al. 2003).

Compared to the present results, higher occurrence of HHV-6 and HHV-7 in the BALF have been reported in other studies (Ross et al. 2001, Jacobs et al. 2003, Neurohr et al. 2005). In the present

study microscopic blood contamination was often present in the BALF samples with HHV-6 or HHV-7 antigen positive cells. Previous studies have used PCR-based methods, and the proportion of BALF samples with microscopic blood contamination has not been reported. As in the present study, the detection of HHV-6 or -7 in the BALF has only seldom been associated with clinical pneumonitis in the previous studies (Jacobs et al. 2003). Thus it is suggested that most of the positive findings of these viruses from the BALF may actually represent the concomitant antigenemia or DNAemia.

5. Antiviral strategies against CMV, HHV-6, and HHV-7

Since high morbidity and mortality are related to CMV after LTx, antiviral prophylaxis against the virus is widely recommended (Zamora et al. 2005). Therefore, the majority of the LTRs in the Studies III and IV received antiviral prophylaxis (excluding those with a R-/D- match), and, consequently, comparison between the LTRs with and those without prophylaxis was not possible. However, two conclusions regarding the prophylaxis may be drawn from the present results:

(i) Intravenous/oral ganciclovir or oral valganciclovir prophylaxis delayed CMV infection when compared to the HTRs without prophylaxis, but CMV infection developed soon after the cessation of the prophylaxis in the Study III. The fact that short-term (≤ 3 months) antiviral prophylaxis delays rather than prevents CMV infection is in concordance with previous studies (Duncan et al. 1994, Soghikian et al. 1996, Humar et al. 2005). This raises a question about the reasonable length of antiviral prophylaxis. After the Study III, the length of the CMV prophylaxis in the LTRs was expanded up to 6-9 months at Helsinki University Central Hospital as can be seen in the Study IV. It is also to be noted that the incidence of CMV antigenemia was 50 % in LTRs with valganciclovir prophylaxis (median of six months), while all of the five patients (100 %) with ganciclovir prophylaxis for three months developed CMV infection. The difference was not statistically

significant, and the change in the induction therapy may act as confounding factor. Nevertheless, the present results are in concordance with the study by Zamora et al. who showed that the use of valganciclovir prophylaxis for a minimum of 180 POD decreases the occurrence of CMV infection when compared to a shorter duration of prophylaxis (Zamora et al. 2004b).

(ii) Antiviral prophylaxis could not prevent the appearance of HHV-6 or -7 antigenemia. HHV-6, unlike CMV, appeared frequently even during the prophylaxis. Razonable and coworkers found a lower incidence of HHV-6DNAemia and a similar incidence of HHV-7DNAemia compared to the historical data in SOT recipients (no LTRs included) receiving CMV prophylaxis (Razonable et al. 2005). A considerable proportion of LTRs (50 %) developed HHV-6DNAemia despite ganciclovir prophylaxis in the study by Jacobs et al., but the incidence was even higher (91 %) among the patients without prophylaxis (Jacobs et al. 2003). Similarly to the studies on the incidence of HHV-6 and HHV-7, the variability in the immunosuppressive medications and virological methods is probably the main reason for the differences between the studies assessing the efficacy of antiviral prophylaxis. Nevertheless, the higher susceptibility of CMV to ganciclovir compared to HHV-6 and HHV-7 in vitro gives evidence of the different efficacy of the antiviral prophylaxis against the three beta-herpesviruses (De Clercq et al. 2001). In the present study, HHV-7 antigenemia was more common in the patients receiving ganciclovir than valganciclovir prophylaxis, but the change in the use of the ATG induction therapy may explain the difference.

Pre-emptive treatment strategy guided by the CMV antigenemia assay was used in both present prospective studies (III and IV). The actual rationale of the pre-emptive strategy and the usefulness of the different CMV assays evaluated in the Study III depend on whether low-level antigenemia is considered significant and warrants the therapy. A wide variation of the cut-off levels for the initiation of the pre-emptive therapy exists between transplant centres and clinical studies (van der Bij and Speich 2001, Vilchez et al. 2001, Kelly et al. 2000, Limaye et al. 2002). Relevant thresholds

are recommended to be validated for clinical practice at each centre (Zamora et al. 2005). In the Study III, there were 17 episodes of spontaneously resolving low-level antigenemia of which DNAemia and mRNAemia were present in only 41 and 12 % of the cases, respectively. It could be argued that using the antigenemia test in the surveillance of CMV with low thresholds for the pre-emptive therapy might lead to unnecessary controls and too aggressive treatment of CMV antigenemia increasing the costs of the pre-emptive treatment strategy, the incidence of the side-effects of antiviral drugs, and the risk for drug resistance of CMV. On the other hand, with no effective treatment available, attention has been focused on the prevention of potential risk factors for the development of BOS and CAV. Some of the recent reports suggest that even asymptomatic CMV antigenemia or DNAemia could lead to chronic allograft injury, which may justify early therapeutic intervention (Potena et al. 2003, Westall et al. 2003, Potena et al. 2006). HHV-6 and HHV-7 antigenemia commonly subsided with the treatment directed against CMV, though the response was somewhat unclear. Nevertheless, the therapeutic dosing of the drugs used to treat established CMV infection may be the main cause why the antiviral therapy seems to be more effective than the prophylaxis against HHV-6 and HHV-7.

The prevention and treatment of CMV infection, possibly together with other beta-herpesviruses, may be of importance, not only in controlling the clinical viral disease, but also in restricting the occurrence of chronic allograft injury in thoracic organ transplant recipients. Whether blocking all CMV replication (low-level antigenemia or DNAemia) or inhibiting HHV-6 or HHV-7 activation can prevent these major complications and improve the long-term survival of the recipients, remains still an unanswered question. Future prospective studies are needed to evaluate the optimal indications for the initiation of the pre-emptive therapy and the overall efficacy of preventive antiviral strategies on CMV, HHV-6, and HHV-7, taking into account both the direct and indirect effects of these viruses.

CONCLUSIONS

Bronchoscopy with BAL, PSB and TBB (LTRs) is a useful and safe diagnostic tool in thoracic organ transplant recipients, when respiratory infection is suspected. Its utility is best at one to six months after Tx when the variability of the microbial aetiology is largest.

The rationale of performing surveillance bronchoscopies on LTRs remains controversial. The surveillance TBBs, in particular, yield only little clinically significant information to support their performance.

P.carinii infections occur in LTRs despite the chemoprophylaxis and in HTRs after discontinuation of the prophylaxis. This justifies the use of bronchoscopy to detect this organism in thoracic organ transplant recipients, even in the era of chemoprophylaxis.

In thoracic organ transplant recipients, CMV DNAemia assay is comparable with the pp65-antigenemia test in guiding the pre-emptive therapy against CMV infections, when threshold levels of over 5 pp65-antigen positive leukocytes/50 000 PMNLs are used as reference. The mRNAemia test is less sensitive than the pp65-antigenemia or the DNAemia test in HTRs and LTRs. This limits its usefulness in the guidance of the pre-emptive therapy.

HHV-6 and HHV-7 antigenemia is common and appears early after LTx. Clinical manifestations are infrequently linked solely to HHV-6 or HHV-7. Antiviral prophylaxis against CMV is not able to prevent the appearance of HHV-6 and HHV-7 antigenemia.

YHTEENVETO (FINNISH SUMMARY)

Elinsiirto tarjoaa loppuvaiheen keuhko- ja sydänsairauksista kärsiville potilaille ennustetta ja elämänlaatua parantavan hoitomahdollisuuden. Keuhkon- ja sydämensiirtopotilaiden hoidossa infektiot ovat merkittävä ongelma yleisyytensä sekä niihin liittyvän korkean kuolleisuuden vuoksi. Elinsiirtopotilailla infektioiden aiheuttajakirjo on laaja ja poikkeaa normaaliväestön vastaavasta kirjosta. Sen vuoksi käyttökelpoiset menetelmät infektioiden diagnostiikassa ovat välttämättömiä. Tässä tutkimuksessa selvitettiin bronkoskopian käyttökelpoisuutta keuhkon- ja sydämensiirtopotilaiden infektiodiagnostiikassa, verrattiin sytomegalovirus(CMV)-infektioiden osoittamiseen käytettäviä menetelmiä näillä potilailla sekä ihmisen herpesvirusten 6 ja 7 (HHV-6 ja HHV-7) esiintymistä ja merkitystä keuhkonsiirtopotilailla.

Tutkimme kaikkien toukokuusta 1988 joulukuuhun 2001 sydämensiirtopotilaille ja helmikuusta 1994 marraskuuhun 2002 keuhkonsiirtopotilaille tehtyjen bronkoskopioiden tulokset. Infektioepäilyn yhteydessä spesifinen diagnoosi saatiin 41 %:lle sydämensiirtopotilaista ja 61 %:lle keuhkonsiirtopotilaista tehtyjen bronkoskopioiden näytteistä. Bronkoskopia osoittautui hyödyllisimmäksi ajanjaksona yhdestä kuuteen kuukautta elinsiirron jälkeen. Sen sijaan oireettomille keuhkonsiirtopotilaille tehdyt ns. seuranta bronkoskopiat toivat harvoin merkityksellisiä löydöksiä, ja hoitoa muutettiin näiden tutkimusten perusteella vain 6 %:ssa tapauksista. Bronkoskopia todettiin varsin turvalliseksi toimenpiteeksi, vaikkakin muutamia vakavia komplikaatioita esiintyi. *Pneumocystis carinii* ja CMV olivat yleisimmät bronkoskopianäytteissä todetut mikrobit molemmissa potilasryhmissä. Yllättävänä löydöksenä voidaan pitää sitä, että *P. carinii* infektioita esiintyi keuhkonsiirtopotilailla myös estolääkityksen aikana.

Seurasimme 17.12.2000 - 15.4.2003 keuhkon- tai sydämensiirron saaneita potilaita verestä tehtävien CMV-antigenemia-, DNAemia- (Cobas Amplicor PCR) ja mRNAemia (NASBA)- testien avulla. CMV-infektiot hoidettiin antigenemiatestin tulosten perusteella, ja kahden muun CMV-testin tuloksia verrattiin antigenemiatestiin. DNAemia-testi oli positiivinen 93 %:lla kaikista lääkehoitoa vaatineista CMV antigenemioista, mutta mRNAemia todettiin vain 61 %:ssa näistä infektioista. Antigenemia- ja DNAemia-testien tulokset korreloivat hyvin aineistossamme. Antigenemiatasoja 2, 5 ja 10 pp65-positiivista leukosyyttiä/50 000 leukosyyttiä vastaavat maksimaalisen sensitivisyyden ja spesifisyyden summan tuottavat DNAemia (sens./spes.)-tasot olivat 400 (75.9 % / 92.7 %), 850 (91.3 % / 91.3 %) ja 1250 (100 % / 91.5 %) kopiota/ml. Edellä mainittuja antigenemiatasoja vastaavat mRNAemia-testin sensitivisyydet olivat vain 25.9 %, 43.5 % ja 56.3 %.

Ajanjaksona 17.12.2000 - 06.10.2004 keuhkonsiirron saaneita potilaita seurattiin CMV-antigenemiatestin lisäksi myös HHV-6:n ja HHV-7:n suhteen valkosolujen antigeenitestien avulla. Keuhkonsiirtopotilaista 91 %:lla todettiin HHV-6 antigenemia ja 50 %:lla HHV-7 antigenemia. Molemmat virukset aktivoituivat yleensä ensimmäisen postoperatiivisten kuukauden aikana. Yhdellä potilaalla HHV-6 viruksen aktivaatio assosioitui radiologisiin keuhkomuutoksiin ja toisella potilaalla neurologisiin löydöksiin, mutta suurimpaan osaan HHV-6 ja HHV-7 antigenemioista ei voitu liittää selkeitä oireita. Gansikloviiri tai valgansikloviiri estolääkityksen aikana 15 potilaalla (79 %) todettiin HHV-6 ja seitsemällä (37 %) HHV-7 antigenemia, mutta vain yhdellä potilaalla CMV antigenemia.

Tutkimuksemme perusteella bronkoskopia on turvallinen ja käyttökelpoinen menetelmä keuhkon- ja sydämensiirtopotilaiden keuhkoinfektioiden diagnostiikassa. Sen sijaan oireettomille keuhkonsiirtopotilaille tehtävien seuranta-bronkoskopioiden hyödyllisyys on kyseenalainen.

Bronkoskopiaa tarvitaan edelleen *P.carinii* infektioiden osoittamisessa. CMV DNAemia-testi (Cobas Amplicor PCR) vaikuttaa toimivan yhtä hyvin CMV infektioiden hoidon ohjannassa verrattuna CMV antigenemiatestiin, kun hoidon aloittamisen kynnysarvoina on käytetty yli 5 pp65-positiivista leukosyyttiä. Sitä vastoin NASBA-testin matala sensitiivisyys rajoittaa sen käyttöä pre-emptiivisen hoidon ohjannassa. HHV-6 ja HHV-7 antigenemia on yleistä keuhkonsiirron jälkeen, eikä CMV infektioita vastaan suunnattu estolääkitys kykene estämään niitä. Näiden virusten aktivaatioon liittyy kuitenkin harvoin selkeitä oireita tai löydöksiä.

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